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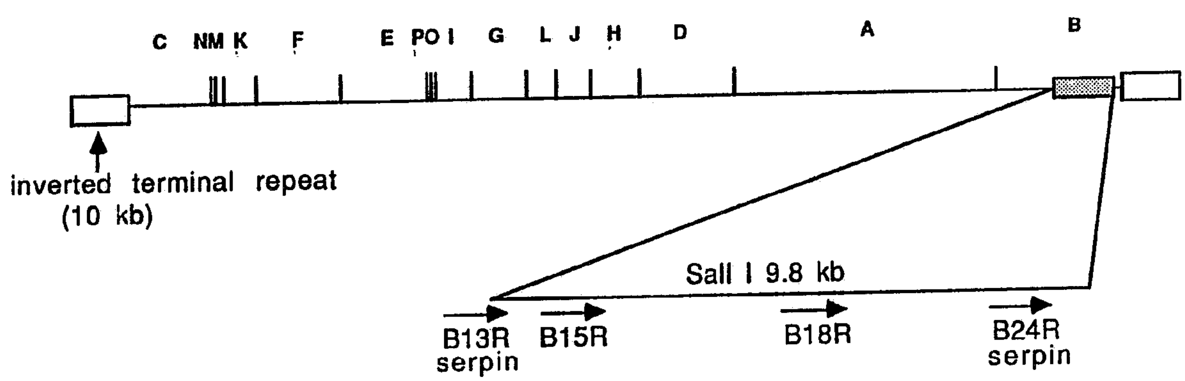

(57) Abstract

This application concerns vaccinia vectors and it is based upon the discovery of three unobvious gene sequences in the viral genome. One or more of the gene sequences may be inactivated, or part of all of one or more of these gene sequences may be deleted from the viral genome to allow (i) greater attenuation of the virus and/or (ii) enhancement of immunogenicity of recombinant vaccinia virus; and/or (iii) further gene sequence insertion sites, so that more foreign DNA may be incorporated in the virus. Where however, the gene sequences are essential for viral replication, viral attenuation can still be effected by altering the gene product (e.g. by manipulation at gene level), such that a protein function effecting pathogenicity is adversely affected, whilst keeping the protein functional for virus replication.

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VACCINIA VECTORS, VACCINIA GENES AND EXPRESSION PRODUCTS THEREOF

The present invention relates to recombinant vaccinia virus vectors. In particular it relates to the attenuation of the virus, to potential enhanced immunogenicity of the virus, to the provision of sites for the insertion of heterologous gene sequences into the virus, and to the use of the recombinant virus vectors thereby provided. It also relates to proteins which are the expression products of vaccinia genes.

Live vaccinia virus was used as the vaccine to immunise against, and eradicate smallpox. Vaccinia virus (VV) is the prototypical member of the poxvirus family and therefore it has been extensively studied. It is a large DNA-containing virus which replicates in the cytoplasm of the host cell. The linear double-stranded genome of approximately 185,000 base pairs has the potential to encode at least 200 proteins (Moss, B. (1990). In "Virology". B.N. Fields Ed. pp 2079-2111. Raven Press, New York.) The cytoplasmic site of replication requires that vaccinia virus encodes many enzymes and protein factors necessary for transcription and replication of its genome. The virus also encodes a variety of factors which modulate virus replication in the multicellular host and aid evasion of the host immune

system (Moss, B. (1990). In "Virology" B.N. Fields Ed. pp 2079-2111. Raven Press, New York). Advances in molecular genetics have made possible the construction of recombinant vaccinia viruses that contain and express genes derived from other organisms (for review see Mackett, M. & Smith G.L. (1986), J. Gen. Virol., 67, 2067-2082). The recombinant viruses retain their infectivity and express the foreign gene (or genes) during the normal replicative cycle of the virus. Immunisation of animals with the recombinant viruses has resulted in specific immune responses against the protein(s) expressed by the vaccinia virus, including those protein(s) expressed by the foreign gene(s) and in several cases has conferred protection against the pathogenic organism from which the foreign gene was derived.

Recombinant vaccinia viruses have, therefore, potential application as new live vaccines in human or veterinary medicine. Advantages of this type of new vaccine include the low cost of vaccine manufacture and administration (because the virus is self-replicating), the induction of both humoral and cell-mediated immune responses, the stability of the viral vaccine without refrigeration and the practicality of inserting multiple foreign genes from different organisms into vaccinia virus, to construct polyvalent vaccines effective against

multiple pathogens. A disadvantage of this approach, is the use of a virus vaccine that has been recognised as causing rare vaccine-related complications.

The applicants have now identified unobvious gene sequences in the viral genome. In the present application, the structure of two genes B15R and B18R from near the right inverted terminal repeat (ITR) are described and shown to encode proteins related to each other, to the immunoglobulin (Ig) superfamily and to the human (Sims, J.E., Acres, R.B., Grubin, C.E., McMahan, C.J., Wignall, J.M. (1989). Proc Natl. Acad. Sci USA 86, 8946-8950) and murine (Sims, J.E., March, C.J.,Cosman, D., Widmer, M.B., MacDonald, H.R., McMahan, C.J., Grubin, C.E., Wignall, J.M., Jackson, J.L., Call, S.M., Friend, D., Alpert, A.R., Gillis, S., Urdal, D.L. and Dower, S.K. (1988). Science 241, 585-589) interleukin-1 receptors (IL-1R). The product of B18R is expressed on the cell surface early during infection and antibodies directed against it confer resistance to virus infection without directly neutralizing virus infectivity (Ueda, Y., Morikawa, S. and Matsuura, Y. (1990). Virology 177, 588-594; Ueda, Y. and Tagaya, I. (1973). J. Exp. Med. 138, 1033-1043; Ikuta, K., Miyamoto, H. and Kato, S. (1980) J. Gen. Virol., 47, 227-232). The applicants propose that either of these gene products may bind interleukin-1 and/or 6 and prevent these cytokines

reaching their natural receptors. In consequence the inflammatory response is diminished and virus replication enhanced. This constitutes a novel method of virus immune evasion. The applicants also disclose herein the structure of a third gene SalL4R. This gene appears to be related to C-type animal lectins in general and to CD23, a cell surface protein expressed on inactivated B lymphocytes. The applicability of SalL4R to virus attenuation is discussed in more detail in the following pages.

The applicants propose that one or more of the gene sequences may be inactivated, or part or all of one or more of these gene sequences may be deleted from the viral genome to allow (i) greater attenuation of the virus; and/or (ii) enhancement of immunogenicity of recombinant vaccinia virus; and/or (iii) further gene sequence insertion sites, so that more foreign DNA may be included in the virus. Where however, the gene sequences are essential for viral replication, viral attenuation can still be effected by altering the gene product (e.g. by manipulation at gene level), such that a protein function affecting pathogenicity is adversely affected, whilst keeping the protein functional for virus replication.

According to one aspect of the present invention there is provided a vaccinia virus vector wherein a) part

or all of one or more of the following nucleotide sequences is deleted from the viral genome; and/or b) one or more of said nucleotide sequences is inactivated by mutation or the insertion of foreign DNA; and/or c) one or more of said nucleotide sequences is changed to alter the function of a protein product encoded by said nucleotide sequence; which nucleotide sequences are sequences designated herein as i) B15R, ii) B18R iii) SalL4R. Mutation of the nucleotide sequence may be effected by the deletion, addition, substitution or inversion of one or more nucleotides.

DNA sequences encoding one or more heterologous polypeptides may be incorporated in the viral genome. The DNA sequences encoding the heterologous peptides may be inserted into one or more ligation sites created by the deletion or deletions from the viral genome. A heterologous peptide is one not normally coded for by wild type vaccinia virus. Typically the heterologous nucleotide sequence will encode an immunogen or a desirable polypeptide product. An immungenic polypeptide will be substantially homologous to an epitope expressed by a pathogenic organism during infection, and which is seen by the infected individual as foreign.

The recombinant vaccinia viruses of the present invention have the potential for enhanced immunogenicity. This may result from either the deletion of vaccinia

genes which cause immunosuppression (eg. the interleukin receptors and the complement homologue and the human FcR for IgE) or by insertion of a gene which potentiates the immune response (e.g. expressing the authentic CD23 gene in vaccinia virus).

Therefore, the present invention provides a vaccinia virus wherein a) part or all of one or more vaccinia nucleotide sequences causing immunosuppression are deleted from the viral genome; and/or b) one or more of said vaccinia nucleotide sequences causing immunosuppression is inactivated by mutation or the insertion of foreign DNA; and/or c) one or more of said vaccinia nucleotide sequences causing immunosuppression is changed to alter the function of a protein product encoded by said nucleotide sequence; which nucleotide sequences are sequences designated herein as i) B15R ii) B18R iii) SalL4R. In particular, the vaccinia nucleotide sequence may be the sequence designated herein as SalL4R.

Where the vaccinia virus comprises a DNA sequence encoding a heterologous polypeptide which potentiates the immune response, the DNA sequence may encode CD23.

The recombinant vaccinia vectors of the present invention may be used as immunogens for the production of monoclonal and polyclonal antibodies or T-cells with specificity for heterologous peptides encoded by DNA sequences ligated into the viral genome. The term

antibody as used above should be construed as also covering antibody fragments and derivatives of a parent antibody and which have the same specificity as the parent antibody.

The invention also provides the monoclonal antibodies, polyclonal antibodies, antisera and/or T cells obtained by use of the recombinant vaccinia vectors provided.

The antibodies produced by the use of the recombinant virus vectors hereof can be used in the diagnostic tests and procedures, for example, in detecting the antigen in a clinical sample;

and they can also be used therapeutically or prophylactically for administration by way of passive immunisation.

Also provided are diagnostic test kits comprising monoclonal antibodies, polyclonal antibodies, antisera and/or T cells obtained by the use of the recombinant vaccinia vectors provided.

Also provided are vaccines and medicaments which comprise a recombinant vaccinia virus hereof. These may have enhanced safety and immunogenicity over current vaccinia virus strains for the reasons indicated.

According to another aspect of the present invention there is provided a polypeptide encoded by a nucleotide sequence selected from those defined above and alleles

and variants of said polypeptides.

The invention also includes sub-genomic DNA sequences encoding such a polypeptide, recombinant cloning and expression vectors containing such DNA, recombinant microorganisms and cell cultures capable of producing such a polypeptide.

The invention also provides a method of attenuating a vaccinia virus vector which comprises: a) deleting part or all of one or more of the following nucleotide sequences from the viral genome; and/or b) inactivating one or more of said nucleotide sequences by mutating said nucleotide sequences or by inserting foreign DNA; and/or c) changing said one or more nucleotide sequences to alter the function of a protein product encoded by said nucleotide sequence; which nucleotide sequences are sequences designated herein as: i) B15R, ii) B18R, iii) SalL4R.

The invention also provides a method which comprises using a vaccinia virus vector as defined herein to prepare a vaccine or a medicament.

The present invention also provides the translation products encoded by the nucleotide sequences B15R and B18R disclosed herein. These translation products may be utilised as anti-inflammatory medicaments.

The invention also provides methods using these translation products for the preparation of an

anti-inflammatory medicament.

BRIEF DESCRIPTION OF THE DRAWINGS

In order that the present invention may be understood more clearly, the identified gene sequences will be described more fully with reference to the figures listed below.

Figure 1. A) HindIII restriction map of the 186 kb VV genome. The 9.8 kb SalI I fragment is expanded to show the position and direction of transcription of the genes B15R and B18R and the serpin genes (Smith, G.L., Howard, S.T. and Chan, Y.S. (1989). J. gen. Virol. 70, 2333-2343) (this nomenclature indicates the genes are the fifteenth and eighteenth orfs starting from the left end of HindIII B and are transcribed rightwards towards the genomic terminus) B) Nucleotide sequence and deduced amino acid sequence of gene B15R. Potential transcriptional control signals are underlined and a possible signal peptide at the N-terminus is boxed. Sites for the addition of N-linked carbohydrate (NXS/T) are boxed and the cys residues likely to form disulphide bonds within Ig domains are stippled. C) Nucleotide sequence and deduced amino acid sequence of gene B18R. The three amino acid positions at which the sequence differs from the published sequence of this gene from another strain of VV (Ueda, Y., Morikawa, S. and Matsuura, Y. (1990). Virology 177, 588-594) are shown. Other features as marked in

(B).

Figure 2. Amino acid alignment of the Ig domains from B15R and B18R with the Ig domains of the human and murine IL-1R, the human IL-6R, the VV haemagglutinin (VV HA), domain 1 of the fasciclin II, domain 3 of myelin-associated glycoprotein and the V-domain of Ig kappa. The regions predicted to form the β-strand structures of Ig domains are indicated above the alignment. Residues identical in 6 or more sequences are boxed. A few residues between the β-strands B and C have been omitted. Also omitted for brevity are β-strands D and, where appropriate, C' and C''. Higher numbers of residues (about 30 or more) between strands C and E are indicative of the V domains.

Figure 3. Amino acid alignment of B15R with the external regions of the IL-1R from human and mouse and the signal sequence and single Ig domain of human IL-6R. Gaps have been introduced to maximise the sequence alignments and are indicated by dashes. Where 4 sequences are aligned, the boxes indicate identical amino acids in three sequences, otherwise boxes indicate complete conservation in all aligned sequences. Potential sites for addition of N-linked carbohydrate are underlined. Arrows and numbers mark the cysteines predicted to form intradomain disulphide bonds of Ig structural units.

Figure 4. Shows the nucleotide sequence and amino acid

sequence for the gene SalF3R.

Figure 5. Shows hydrophobicity profiles of vaccinia virus genes SalL4R and SalF3R (renamed SalF2R, Smith, G.L., Chan, Y.S. & Howard, S.T. J. Gen. Virol. 72, 1349, 1991). Vertical lines on the horizontal axis indicate blocks of 20 amino acids.

Figure 6. Shows the amino acid alignment of the vaccinia virus SalL4R gene (VV L4R) with similar lectin-like molecules from vaccinia virus (VV F3R encoded by SalF3R), fowlpox virus FPV 2, 8 & 11 (Tomley et al., J. gen. Virol. 69, 1025 (1988)), the human low affinity receptor for IgE (HFcR) (Kitutani et al., Cell 47, 657, (1986)), the antifreeze polypeptide from Hemitripterus americans (AFP) Ng et al., J. Biol. Chem. 261, 15690 (1986)) and a lectin from Megabalanus vosa (acorn barnacle) (ABLec) (Maramoto and Kamiya, Biochem. Biophys. Acta., 874, 285 (1986)). Residues conserved in six or more sequences are boxed.

Figure 7. Shows the nucleotide sequence and deduced amino acid sequence of the SalL4R gene. Potential transcriptional control signals are underlined, and the experimentally determined initiation site for late RNA is shown by ****. The potential site for addition of N-linked carbohydrate is boxed (NST), as is a hydrophobic N-terminal signal peptide. The nucleotide sequence shown maps from positions 1755-2498 from the left end of the

SalI L fragment of vaccinia virus strain WR.

Figure 8. S1 nuclease protection analysis of mRNA from genes B15R (Figure 8A) and B18R (Figure 8B). The probes used as described in the text. Lanes 1-4 are an M13 squencing ladder, lane 5 shows the probe used for hybridization, and lanes 6-8 show the DNA fragments protected by hybridization with tRNA, early, or late RNAs, respectively.

Figure 9. Identification of the B15R gene product as a Mr 50 K secretory glycoprotein. Cells were infected with either WT virus (lanes 2, 3, 7 & 8) or recombinant virus vAA1 (lanes 4, 5, 9 & 10) or mock infected (lanes 1 & 6) and labelled with [35S]-methionine either early (lanes 2, 4, 7, & 9) or late (lanes 3, 5, 8, & 10) during infection. Lanes 1-5 show cell extracts & 6-10 supernatants. Samples were run on a polyacrylamides gel and an autoradiograph prepared. The position of the Mr 50 K protein in the supernatant of vAA1 infected cells is indicated by an arrow.

Figure 10. Plaque size in the presence or absence of IPTG. BSC-1 monolayers were infected with WT, vSAD7 or vSAD9 in the presence or absence of IPTG. 2 days later the cells were stained with crystal violet and photographed.

Figure 11. BSC-1 cells were infected with either vSAD7 or vSAD9 in the presence or absence of IPTG. Infections

were at either 10 plaque forming units (pfu) per cell (panels A & B) or 0.001 pfu / cell (panels C & D). Virus present within infected cells at the indicated times after infection was determined by plaque assay on fresh duplicate monolayers of BSC-1 cells.

Figure 12. The morphogenesis of vSAD9. BSC-1 cells infected at 25 pfu/cell with vSAD9 with or without 5 mM IPTG were incubated for 6, 12 and 24 hours before being fixed with 2% gluteraldehyde, embedded, sectioned and observed by electron microscopy. (A) Section of a cell 24 hpi in the absence of IPTG showing virus factories and INV particle (4). 1-3 as for (B). Magnification 5800 X. (B) Detail of vSAD9 virus factory without IPTG at 24 hpi: (1) partially formed lipid crescents, (2) early immature particle completely surrounded by lipid, (3) immature particle containing condensed nucleoid. Magnification 36,000 X. (C) Mature INV, indicated by arrow, produced in the absence of IPTG, 24 hpi. Magnification 36,000 X. (D) Mature INV produced in the presence of IPTG, 24 hpi. Magnification 36,000 X. (E) Cross section of a microvillus of a vSAD9-infected cell in the presence of IPTG 24 hpi showing multiple INV surrounded by double-membranes, example indicated by arrow. Magnification 36,000 X. (F) Cell 24 hpi in the presence of IPTG: (1) INV wrapped in two layers of Golgi-derived membrane, (2) egress of EEV after the outer membrane

surrounding a mature virus particle has fused with the plasma membrane of the cell. Magnification 72,000 X.

Figure 13. Immunoblot analysis of the SalL4R glycoproteins. Cells were infected for 24 hours with WR (lanes 1 and 2), or vSAD9 (lanes 3-6), or mock infected (lanes 7 and 8), in the presence (+) or absence (-) of 5 mM IPTG and/or 1 μg/ml tunicamycin as indicated. Extracts were resolved by SDS-PAGE, transferred to nitrocellulose, incubated with anti-SalL4R serum and immune complexes detected using alkaline phosphatase-conjugated donkey anti-rabbit Ig (materials and methods). The molecular weights of the observed proteins are shown in kDa.

Figure 14. Carbon coated copper 400 mesh grids were floated on 4 x 107 pfu of IHD-J INV (panel D) or EEV (panels A-C, E & F) purified virus particles in 96 well tissue culture dishes. Grids were washed with PBS and TBS for 2 mins, and then in 50% ethanol for 30 secs. Virus-coated grids were then incubated for 15 mins in TBG (TBS pH 8.2, 0.1% BSA [Fraction V], 1% gelatin), before being transferred to wells containing either anti-SalL4R (Panels A & C-E), or an unrelated rabbit serum diluted 1/50 in TBS containing 1% BSA (panel F), or affinity purified anti-SalL4R specific Ig diluted 1/5 in the same (panel B). After 50 mins incubation at room temperature, virion-coated grids were washed for 10 mins in TBG, and

bound Ig detected by incubation for 30 mins in colloidal gold-conjugated goat anti-rabbit Ig diluted 1/10 in TBS containing 1% BSA. Grids were washed for 5 mins with TBG and then TBS, before proteins were fixed in TBS containing 2% gluteraldehyde. Virus particles were finally negatively stained using 2% uranyl acetate.

All the genetic manipulations described below were carried out according to standard procedures (Molecular cloning, eds. Sambrook, Fritsch & Maniatis, Cold Spring Harbor Laboratory Press, 1989) and the conditions used for enzymatic reactions were as recommended by the manufacturer (GIBCO-BRL Life Technologies).

Determination of the Nucleotide and Amino Acid Sequences.

The nucleotide sequence of the SalI I and Sal I L restriction fragment of the vaccinia virus genome (strain WR) were determined by established methods (Sanger, F. et al. (1980), J. Mol. Biol., 143, 161-178) and Bankier, A. and Barrell, B.G. (1983) Techniques in Life Sciences B508., 1-34, Elsevier.

For example, the 9.8 kb SalI I fragment of vaccinia virus (strain WR) was isolated from cosmid 6, which contains virus DNA derived from a rifampicin resistant mutant (Baldick, C.J. & Moss, B. (1987) Virology 156, 138-145), and was cloned into SalI cut pUC13 to form plasmid pSalI I. The SalI fragment was separated from plasmid sequences and self-ligated with T4 DNA ligase.

Circular molecules were randomly sheared by sonication, end-repaired with T4 DNA polymerase and Klenow enzyme and fragments of greater than 300 nucleotides cloned into SmaI cut M13mp18. Single stranded DNA was prepared and sequenced using the dideoxynucleotide chain termination method (Sanger, F., Nicklen, S. & Coulson, A.R. (1977) Proc. Natl. Acad. Sci. USA, 74, 5463-5467), using $[^{35}S]$-dATP and buffer gradient polyacrylamide gels (Biggin, M.D., Gibson, T.J. & Hong, G.F. (1983), Proc. Natl. Acad. Sci. USA, 80, 3693-3695). For further details see (Bankier, A.T., Western, K.M. & Barrell, B.G. (1987) in Wu R. (ed.) Methods in Enzymology 155, 51-93. Academic Press, London). The 6.3 kb SalI L fragment was similarly treated.

Computer analysis

Nucleotide sequence data were read from autoradiographs by sonic digitiser and assembled into contiguous sequences using programmes DBAUTO and DBUTIL (Staden, R. (1980) Nucleic Acids Res. 8, 3673-3694; Staden, R. (1982) Nucleic Acids Res. 10, 4731-4751) on a VAX 8350 computer. The consensus sequence was translated in 6 frames using programmes ORFFILE and DELIB (M. Boursnell, Institute of Animal Health, Houghton, UK.). Open reading frames were compared against SWISSPROT protein database version 14 and against the applicants own database of vaccinia amino acid sequences using

programme FASTP (Lipman, D.J. & Pearson, W.R. (1985) Science 227, 1435-1441). Alignments of multiple protein sequences were performed using programme MULTALIGN (Barton, G.J. & Sternberg, M.J.E. (1987) J. Mol. Biol. 198, 327-337).

There follows a detailed description of the individual gene sequences B15R, B18R and SalL4R the applicants have identified.

Genes B15R and B18R

Genes B15R and B18R (Figure 1) from near the right hand inverted terminal repeat (ITR) are predicted to encode proteins of 36.5 kDa and 40.7 kDa, respectively, that have an N-terminal hydrophobic sequence, possible attachment sites for N-linked carbohydrate and hydrophobic residues near the C-terminus. These properties are consistent with the mature proteins being either virion, cell-surface or secretory glycoproteins.

The nucleotide sequence and deduced amino acid sequence of the gene designated B15R is shown in figure 1B. The nucleotide sequence shown for B15R is 11462-12664 nucleotides from the left end of the vaccinia virus HindIII B fragment and the coding region is at nucleotide positions 11584-12561 (or at nucleotides 815 to 1792 from the left end of the SalI I fragment). Similarly, the nucleotide sequence and deduced amino acid sequence of the gene designated B18R is shown in figure

1c. The nucleotide sequence shown for B18R is 15448-16741 nucleotides from the left end of the vaccinia virus HindIII B fragment and the coding region is at nucleotide positions 15448-16741 (or at nucleotides 4799 to 5851 from the left end of the SalI I fragment).

The single letter code is used for the designation of amino acids.

B15R and B18R each possess three domains with characteristics of the immunoglobulin (Ig) superfamily (Williams, A.F. and Barclay, A.N. (1988). Ann. Rev. Immunol. 6, 381-405) namely a pair of cysteines forming an intradomain disulphide bridge, sequences predicted to form β-strand structures and an invariant tryptophan in β-strand C. In B15R these cysteines are present at positions 48 and 99, 143 and 194, and 242 and 309. In B18R the corresponding cysteines are at positions 73 and 129, 172 and 221, and 272 and 333. The distance between these cysteine pairs in B15R (51, 51 and 67 residues) suggest the first two domains are C regions while the third maybe a V-domain. For B18R the distances (56, 49 and 61) suggest these are C-domains. These regions are aligned with selected Ig domains of IL-1R (interleukin 1 receptor and interleukin 6 receptor), VV haemagglutinin and Ig kappa (Hilschman, N. and Hoppe-Seyer's, Z. (1967) Physiol. Chem. 348, 1077-1080), fasciclin II (Harrelson, A.L. and Goodman, C.S. (1988). Science 242, 700-708),

chicken neural cell adhesion molecule (NCAM) (Hemperley, J.J., Murray, B.A., Edelman, G.M. and Cunningham, B.A. (1986). Proc. Natl. Acad Sci. USA 83, 3037-3041) and myelin-associated glycoprotein (Salzer, J.L., Holmes, W.P. and Colman, D.R. (1987). J. Cell Biol. 104, 957-965) (Figure 2). In this alignment the β-strands C', C'' and D have been omitted for brevity. The cysteines forming the intradomain disulphide bridge and the tryptophan in β-strand C are completely conserved. The relationship between the vaccinia proteins and the Ig family was confirmed by statistical computational analysis using the programme ALIGN (Dayhoff, M.O., Barker, W.C. and Hunt, L.T. (1983). Meth. Enzymol. 91, 524-545) (Table 1). With B15R the highest individual scores are found against he human and murine IL-1R domains. Domain 2 of B18R also scores well against the IL-1R domains and overall there are highly significant scores against a wide range of Ig domains.

An alignment of B15R with the extracellular regions of IL-1Rs and IL-6R (Figure 3) and the above alignment of individual domains (Figure 2) indicates a closer relationship between IL-1Rs, IL-6R and the VV Ig domains than other Ig members. This exemplified by the following observations.

(1) B15R and the external region of IL-IRs have a very

similar length.

(2) There are additional conserved cysteines in B15R, B18R and the IL-1Rs located near the beginning of β-strands A and G in domain 1 and at similar positions in domain 2 of the IL-1Rs and B18R. These cysteines lie within the 3-dimensional structure of an Ig C domain in positions probably allowing another intradomain disulphide bond.

(3) In B15R, both IL-1Rs and IL-6R there is a proline following the invariant cysteine in β-strand B of domains 1 and 2, an unusual residue in this position. B18R domain 1 also contains proline at this position.

(4) In β-strand F of domain 3 of B15R and both IL-1R sequences, the glycine typical of other Ig domains is absent. Moreover, the otherwise invariant tyrosine is replaced in both IL-1Rs and in B15R with phenylalanine.

(5) A glycosylation site is conserved in domain 1, β-strand F of IL-1Rs and B15R despite divergence of amino acid sequence.

(6) Domain 3 does not contain additional cysteines and is longer than 1 and 2 in B15R, B18R and the IL-1Rs.

Very recently the sequences of additional (type II) human and murine interleukin-1 receptors were reported (McMahan et al., EMBO J. 10, 2821-2832, 1991). These are more closely related to the vaccinia virus B15R and B18R genes than are the formerly described type I IL-1

receptors. Indeed vaccinia B15R is more closely related to human and murine type II IL-1 receptors than either of these are to the type I IL-1 receptors. Since both type I and II IL-1 receptors have been shown to bind IL-1, it is very probable that the products of either B15R and/or B18R will also bind this cytokine.

To determine if the genes B15R and B18R are expressed within vaccinia virus infected cells, mRNA corresponding to these genes was analysd by S1 nuclease protection experiments. The radiolabelled probe used for detection of B15R mRNA was prepared by cloning the SalI-XbaI fragment from the left end of the vaccinia virus SalI I restriction fragment into pUC118. This plasmid was used as a template for polymerase chain reaction using an oligonucleotide complementary to the coding strand of B15R and the universal primer complementry to pUC118. The PCR product was purified, labelled with y[32]-ATP using polynucleotide kinase, digested with SalI I, and a 1024 bp fragment isolated. This was hybridized to early or late virus mRNA, hybrids mechanism employed by VV against the host immune response. Other mechanisms, proposed or proven, include the interference with the complement system by a secretory homologue of C4b binding protein (Kotwal, G.J. and Moss, B. (1988). Nature 335, 176-178) the expression; of serine protease inhibitors which may block the

presentation of peptides to cytotoxic T cells (Boursnell, M.E.G., Foulds, I.J., Campbell, J. and Binns, M.M. (1988). J. gen. Virol. 69, 2995-3003; Smith, G.L., Howard, S.T. and Chan, Y.S. (1989). J. gen. Virol. 70, 2333-2343) and restrict the infiltration of infected lesions with leukocytes (Palumbo, G.J., Pickup, D.J., Fredrickson, T.N., McIntyre, L.J. and Buller, R.M.L. (1989), Virology, 172, 262-273).

Protein sequence comparisons show that these VV proteins are related to each other (22.5% identity), to the human and murine IL-1R, the human IL-6R (Yamasaki, K., Taga, T., Hirat, Y., Yawata, H., Kawanishi, Y., Seed, B., Taniguchi, T., Hirano, T and Kishismoto, T. (1988). Proc. Jpn. Acad, 64, 209-211) and the immunoglobulin (Ig) superfamily (Williams, A.F. and Barclay, A.N. (1988). Ann. Rev. Immunol. 6, 381-405). Members of this family contain varying numbers of structurally similar domains (Ig domains) and perform diverse functions, although a unifying theme is surface interations between cells or by binding cytokines. The VV haemagglutinin is another member of this superfamily (Jin, D., Li, Z., Jin, Q., Yuwen, H. and Hou, Y. (1989). J. Exp. Med. 170, 571-576). The B18R sequence from VV strain IHD was recently reported but the relationship to interleukin receptors and the Ig superfamily was not described (Ueda, Y., Morikawa, S. and Matsuura, Y. (1990). Virology 177,

588-594).

The present applicants have identified herein the nucleotide and amino acid sequence data for B15R and B18R and identified the surprising homology between the gene products of B15R and B18R to human and murine IL-1R, human IL-6R and the immunoglobulin (Ig) superfamily.

Cytokines IL-1 and IL-6 by binding to their respective natural receptors mediate immune responses against an invading pathogen and cause inflammation. VV appears to be combatting this part of the immune response by producing proteins which mimic the receptors for IL-1 and IL-6. Thus the function of these VV proteins appears to be one of binding the cytokines to prevent them reaching their natural receptors. In this way the virus reduces the inflammatory response directed against it. Thus the virus is able to replicate more effectively. The applicants proposal is to use this surprising observation to render vaccinia virus less harmful, so that problems associated with its use as a vaccine and/or other problems are ameliorated. A recent report (Perkus, M, Goebel, S.J., Davis, S.W., Johnson, G.P., Norton, E.K. and Paoletti, E. (1991). Virology 180, 406-410) indicates that large near-terminal sections of the genome of the Copenhagen strain of vaccinia virus are non-essential for virus replication in vitro, and comparison of the nucleotides sequences of B15R and B18R

presented here with those reported to be non-essential for the Copenhagen strain (Goebel, S.J., Johnson, G.P., Perkus, M, Davis, S.W., Winslow, J.P. and Paoletti, E. (1990). Virology 179, 247-266) indicates that WR genes B15R and B18R are included in the non-essential regions. However, it remains to be established whether either or both of these genes are non-essential for replication of the WR strain of virus. Deletion of these ORFs could provide a suitable means of virus attenuation.

Since the translation products encoded by the nucleotide sequences B15R and B18R appear likely to bind interleukin 1 or interleukin 6, these protein products will be useful as antiinflammatory agents. The proteins may be produced in a recombinant system according to techniques well known in the art. Thus the nucleotide sequences provided herein could be inserted into a suitable expression vector (not necessarily vaccinia). That vector can then be used to transform a cell line suitable for the production of these particular proteins.

<u>SalL4R</u>

The present applicants have already filed a patent application (PCT/GB90/00493) disclosing SalF3R (renamed SalF2R, Smith et al., J. Gen Virol. <u>72,</u> 1349-1376, 1991), a gene related to SalL4R. The nucleotide sequence and deduced amino acid sequence of the gene designated SalF3R is shown in figure 4. The single letter code is used for

the designation of amino acids. The coding region of the SalF3R gene maps between nucleotides 595 and 1071 from the left end of the SalIF fragment. The molecular weight of the primary translation product is predicted to be 18.1 kiloDaltons (kD). Near the amino terminus there is a string of hydrophobic amino acids thought to cause the protein to be associated with, or secreted through, the cell membrane. Near the carboxy terminus there are three potential N-linked glycosylation sites, indicating that the mature gene product is a glycoprotein.

Surprisingly, the present applicants have now identified the gene designated herein as SalL4R which shows homology to, and appears to be structurally related to SalF3R, to C-type animal lectins in general and to CD23. The gene SalL4R maps 1755-2498 from the left hand end of SalIL fragment of the vaccinia virus strain WR.

Comparisons of the deduced amino acid sequences SalF3R shown in figure 4, and of SalL4R shown in figure 8, with the protein database SWISSPROT established several significant homologies. These are shown in figure 6. The amino acid sequence encoded by the genes SalF3R and SalL4R show sequence homology to a variety of lectins and the nearest homologue (for SalF3R) is human CD23 (see later). In particular, the amino acid sequence encoded by the genes SalF3R and SalL4R show sequence homology with the amino acid sequence of the human low

affinity Fc receptor for IgE (Kitutani, . et al. (1986), Cell, 47, 657), the amino acid sequence of an antifreeze polypeptide from Hemitripterus americans (see Ng, N.F. et al, (1986), J. Biol. Chem., 261, 15690-5) (5) and the amino acid sequence of a lectin from Megabalanus rosa (acorn barnacle) (Maramoto, K. & Kamiya, H. (1986), Biochem. Biochem. Biophys. Acta., 874, 285-295.). SalF3R has a 26.1% amino acid identity over a 92 amino acid region of the human low affinity Fc receptor (FcR) for IgE, 22.4% amino acid identity over a 98 amino acid region of the antifreeze polypeptide from Hemitripterus americans, and a 27.0% amino acid identity over a 63 amino acid region of the lectin from Megabalanus rosa. SalF3R and SalL4R show sequence homology with respect to each other (Fig 6) and similar hydrophobicity profiles (Fig. 5).

The homologies suggest that the proteins encoded by these genes function as lectins or as homologues of the human low affinity FcR for IgE. The latter homology is particularly important, as the human low affinity FcR for IgE is the same as CD23, a cell surface protein expressed on activated B lymphoctyes which is of central importance in regulating B cell growth (Gordon, J. & Guy G.R. (1987), Immunol. Today, 8, 339).

Thus, the vaccinia virus protein encoded by SalF3R or by SalL4R may function as an agonist of the normal

CD23 molecule, to restrict the growth and/or differentiation of B cells and thereby reduce the host immune response to infection by the virus. Deletion of this gene from the virus genome would therefore enhance the host immune response to the virus. The consequence of this could be further restriction of virus growth and hence attenuation. It is also possible that the immune response to foreign proteins expressed by recombinant vaccinia viruses lacking this gene would be enhanced and the efficacy of such candidate vaccines improved. Expression of the authentic human CD23 protein in vaccinia recombinants that do or do not contain the vaccinia homologue of CD23 may also enhance the immunogenicity of recombinant vaccinia virus vaccines that express antigens from heterologous pathogens.

If the protein has alternative or additional functions as a lectin, it may play a role in the attachment of virus to the target cell. Thus, deletion of the functioning gene in this capacity results in virus attenuation since the ability of virus particles to infect cells would be diminished.

A mutant virus with the coding region of the SalF3R gene interrupted and partially deleted has been constructed. A plasmid, pPROF was constructed by the ligation of the leftmost 3524 bp (SalI-EcoRI DNA fragment) of the vaccinia virus SalIF fragment into pUC13

that had been digested with EcoRI and SalI. This plasmid contains the entire coding region of SalF3R and was digested with NsiI, which cuts twice only, within the coding sequence (Figure 6). The digested DNA was treated with bacteriophage T4 DNA polymerase to create blunt ends, and the larger of the two fragments was purified by agarose gel electrophoresis. This fragment was ligated with a gel-purified DNA fragment containing the E.coli xanthine guanine phosphoribosyl transferase (Ecogpt) gene joined to the vaccinia virus 7.5K promoter sequence. The latter fragment was obtained by digestion of plasmid pGpt07/14 (Boyle, D.B. and Coupar, B.E.H. Gene 65, 123-8 (1988)) with EcoR1, followed by treatment of the digested DNA with DNA polymerase (Klenow fragment) to create blunt ends and isolation of a 2.1kb DNA fragment. The ligated DNA was cloned into E.coli, and the resulting bacterial colonies screened for the presence of the desired plasmid with appropriate restriction enzymes. Through this procedure, a plasmid, pSAD3G, was isolated in which 100bp of the SalF3R coding sequence was replaced by a functional copy of the Ecogpt gene under the control of the vaccinia virus 7.5K promoter.

Plasmid pSAD3G was transfected into CV-1 cells that were infected with wild type (WT) vaccinia virus and the virus progeny derived from these cells after 48 hours at 37°C were then plated on fresh CV-1 cells in the presence

of mycophenolic acid (MPA), xanthine and hypoxanthine. These drugs permit the replication only of recombinant viruses which contain and express the Ecogpt gene (Boyle & Coupar 1988 supra; Falkner & Moss, J. Virol, 62, 1849-54, 1988). After three rounds of plaque purification, the virus was amplified in larger cultures of CV-1 cells. Southern blot analysis of virus DNA confirmed that the Ecogpt gene was present at the predicted location in the virus genome, that no functional copy of the SalF3R gene remained and that no other virus genomic DNA re-arrangements had occurred. Since a virus lacking the SalF3R gene is viable, these data established that the gene SalF ORF3 is non-essential for virus replication in vitro.

The likelihood that the inactivation of the SalF3R coding region would generate an attenuated virus, depends on whether or not the region is expressed during normal virus replication. To address this point, virus mRNA transcribed from this region during the early and late phase of infection was analysed by Northern blotting. A single-stranded radio-labelled DNA probe complementary only to the coding strand of SalF3R detected an early mRNA species of about 600 nucleotides. Late during infection, this mRNA was replaced by some RNA species of heterogeneous length which appear as a smear on the Northern blot. Due to the heterogeneous length of late

vaccinia virus mRNA, it is possible that this represents either mRNA initiating from the SalF3R promoter or from further upstream. This data allows the conclusion that the gene SalF3R is certainly transcribed early and possibly also late during infection.

Similar studies have been carried out with SalL4R. Analysis (by S1 nuclease protection experiments) of mRNA isolated from virus infected cells demonstrated that late during infection RNA is transcribed from the TAAATG motif at the 5' end of the SalL4R gene. A virus expressing only an IPTG-inducible form of SalL4R has been constructed in a similar manner to that described for the vaccinia virus 14K protein (Rodriguez and Smith, Nucleic Acids Reserach 18, 5347-5351, 1990). Briefly, a copy of the SalL4R open reading frame was constructed by polymerase chain reaction and cloned into plasmid pPR34 (Rodriguez and Smith, Virology 177, 239-250, 1990) downstream of the IPTG-inducible vaccinia virus 4b promoter. This plasmid was transfected into cells infected with WT vaccinia virus strain WR and a thymidine kinase negative virus (vSAD7) isolated that contains the IPTG-inducible copy of the SalL4R gene within the TK locus. Next a 1.9 kb SpeI DNA fragment from positions 1050-2993 from the left end of the SalI L fragment was isolated and ligated into plasmid pUC13 that had been digested with XbaI to form plasmid pSAD2. This plasmid

was amplified in E. coli, purified and then digested with NaeI that cuts 53 bp downstream of the 5' end of the SalL4R gene coding region. The DNA was then partially digested with ScaI and a linear DNA fragment isolated that had been cut with ScaI at a position 40 bp upstream of the 3' end of the coding region. This DNA fragment was then ligated with a DNA fragment containing the E.coli gpt gene linked to the vaccinia virus 7.5K promoter that had been isolated and rendered blunt-ended as described above for gene SalF3R. The resultant plasmid (termed pSAD8) has 415 bp of Sal4R deleted and replaced with the Ecogpt gene. This plasmid was amplified in E. coli, purified and then transfected into CV-1 cell infected with vaccinia virus vSAD7. Recombinant viruses were selected in the presence of IPTG and mycophenolic acid, and susequently plaque purified and amplified. A recombinant virus which had the IPTG-inducible form of the SalL4R gene within the TK gene locus and the endogenous copy of SalL4R replaced by Ecogpt was called vSAD9. Analysis of the genomic DNA of vSAD7 and vSAD9 by Southern blotting confirmed these viruse had the predicted structures.

If the virus vSAD9 grew normally in the absence of IPTG, i.e. without expression of the SalL4R gene, the encoded protein would be non-essential for virus replication. If, on the other hand, the virus was

dependent on IPTG the SalL4R gene would be essential for virus replication. Figure 10 shows that in the absence of IPTG virus vSAD9 forms tiny plaques while WT virus and vSAD7 form normal size plaques. Upon IPTG induction the plaque size of vSAD9 increases to normal. This indicated that the vSAD9 was unable to spread without SalL4R expression. This was also addressed by following the growth kinetics of vSAD9 with or without IPTG. Figure 11 shows that in cells infected at high multiplicity of infection (moi) the production of intracellular virus was unaffected by the presence or absence of IPTG, and the growth kinetics were indistinguishable from vSAD7. This demonstrates that the SalL4R gene is not required for the production of infectious intracellular virus. However, infections at low moi showed that although the production of INV was not altered during the first 12 hours by the presence or absence of IPTG, thereafter the yields of INV were reduced if IPTG was not included. This indicated that the SalL4R gene is required for the efficient spread of virus to uninfected cells. This conclusion was directly supported by electron microscopic analysis of virus maturation within infected cells (Figure 12). In the absence of IPTG, panels A-C, virus morphogenesis is normal up to the production of INV particles. However, unless IPTG was present no budding of these INV particles in Golgi membrane and egress from the cell was observed.

Panels E & F show this process occurring in the presence of IPTG. Collectively, these data show that the SalL4R gene product is not required for the production of INV but is needed for the envelopment of INV by Golgi-derived membrane and for the egress of EEV particles from the cell.

The SalL4R gene product was identified by raising a polyclonal antisera to a region of SalL4R expressed as a TrpE-fusion protein in E.coli. A 321 bp AccI fragment was isolated from within the Sal4R gene and cloned into plasmid pATH3 (Koerner et al., Meth. Enzymol. 194, 477-490, 1991) to form pSAD24. Expression of the fusion protein was induced in bacteria harbouring pSAD24 with indoleacrylic acid. The digested with S1 nucleae and the products run on a sequencing gel against an M13 ladder. The result (Figure 8A) shows that the B15R gene is transcribed late during infection from a TAAAATG motif at the beginning of the open reading frame.

A probe for analysis of B18R mRNA analysis was produced by digesting pUC118 containing the SalI-XbaI region of vaccinia virus SalI fragment (above) with EcoRI and purifying a 500 bp fragment. This was dephosphorylated with calf intestinal alkaline phosphatase, labelled with g[32]-ATP using polynucleotide kinase, digested with XbaI and a 474 bp fragment isolated. This was hydridized with virus mRNA as for the

B15R probe above. The result (Figure 8B) shows that the B18R gene is transcribed early during infection from upstream of the B18R gene. These data are consistent with primer extension analyses of the mRNA from this gene (Ueda et al., Virology 177, 577-588, 1990).

Direct evidence has been obtained that B15R encodes a secretory glycoprotein of Mr 50 K. A recombinant vaccinia virus (vAA1) was constructed that contained a second copy of the B15R ORF driven from the late p4b promoter. This virus was formed by cloning the B15R ORF into plasmid pRK19 (Kent, R.K. Isolation and analysis of the vaccinia virus p4b gene promoter. PhD Thesis, University of Cambridge, 1988) downstream of the strong late vaccinia 4b promoter. The resultant plasmid was transfected into cells infected with wild type (WT) vaccinia virus (strain WR) and a recombinant virus, that contained the second copy of B15R inserted into the TK gene locus, selected by plaque assay on human thymidine kinase negative cells in the presence of bromodeoxyuridine. BSC-1 cells infected with either vAA1 or WT virus were labelled with [35S]-methionine early (2-4 hours post infection in the presence of cytosine arbinoside) or late (6-8 hours post infection) and the radiolabelled proteins resolved by polyacylamide gel electrphoresis and detected by autoradiography. Figure 9 shows the presence of a glycosylated extracellular

protein of approximately Mr 50 K in the supernatant of cells infected with vAA1 but not WT virus. This protein represents the product of the B15R gene.

It has been reported that the B18R protein can be found on the cell surface early during infection, (Ueda, Y., Morikawa, S. and Matsuura, Y. (1990). Virology 177, 588-594) despite the C-terminal hydrophobic residues being predicted to form the final β-strand of an Ig domain and therefore unlikely to function as a transmembrane anchor. This localization may be mediated by protein-protein interactions possibly involving intermolecular disulphide bonds between additional cysteines (as occurs with other members of the Ig superfamily eg. the immunoglobulins). Alternatively, these cysteines may form intradomain disulphide bridges between the A and G β-strands. Similar arguments apply for B15R. Irrespective of whether the proteins remain on the cell surface or are released, a likely function for either protein is to bind and sequester IL-1R or IL-6R at the site of tissue damage during virus infection. These cytokines would then be unable to reach their natural receptors, resulting in a diminished inflammatory response and an increased ability of the virus to spread and cause disease in the mammalian host. Blockade of the IL-1R can directly attenuate the host inflammatory response (Gershenwald, J.E., Fong, Y., Fahey, T.J.,

Calvano, S.E., Chizzonite, R., Kilian, P.L., Lowry, S.F. and Moldawer, L.L. (1990). Proc Natl. Acad. Sci. USA 87, 4966-4970).

Another likely function for B15R and B18R proteins, is to facilitate virus spread by mediating the interaction of virus particles or infected cells with other cells. There is ample precedent for such a cell-cell interaction with members of the Ig superfamily (eg. MHC antigens, NCAM (Hemperley, J.J., Murray, B.A., Edelman, G.M. and Cunningham, B.A. (1986). Proc. Natl. Acad Sci. USA 83, 3037-3041) and the intercellular protein amalgam (Seeger, M.A. Haffley, L. and Kaufman, T.C. (1988). Cell 55, 589-600). Nonetheless, the closer homology to IL-1R and IL-6R make the binding of cytokines more likely. Indeed, antibodies to the B18R gene product restrict virus replication without neutralising virus infectivity and confer resistance after passive transfer of immune serum (Ueda, Y. and Tagaya, I. (1973). J. Exp. Med. 138, 1033-1043). These observations are consistent with the B18R protein binding cytokines (IL-1) or IL-6) and preventing these mediating their normal function in cells bearing their receptors. Antibody to B18R would block the sequestering of these cytokines so a normal inflammatory response would ensue and virus replication would be restricted. The B15R protein may function in a similar manner.

Such a mechanism constitutes a novel defense fusion protein was purified from inclusion bodies and used to immunise rabbits. This sera was used in western blot analyses of protein extracts from BSC-1 cells infected with WT virus, or vSAD9, or mock infected in the presence or absence of IPTG and tunicamycin. Figure 13 shows that in WT infected cells there are 3 polypeptides of Mr 22, 23 and 24 K detected by the anti-SalL4R serum. These same proteins are seen in cells infected with vSAD9 in the presence of IPTG, but are absent if IPTG is omitted, and from mock infected cells. In the presence of tunicamycin (an inhibitor of N-linked glycosylation) the bands are replaced by a Mr 19 K precursor. This indicates that the primary translation product is glycosylated at the single site for N-linked carbohydrate near the C-terminus of the protein.

The pattern of the proteins encoded by SalL4R are very similar to a group of proteins shown to be components of the EEV envelope (Payne, L.G. J. Virol. 31, 147-155, 1979). This observation together with the requirement for the gene products of SalL4R for virus envelopment and egress, suggested that the SalL4R was present in EEV. This was demonstrated by immunoelectron microscopy. Figure 14 indicates that the antibody to the SalL4R-fusion protein reacts with purified EEV particles (panels A-C, & E) but not INV (panel D). This specific

binding did not require membrane permeabilization (panel E) and was present after immunoaffinity purification of the anti-SalL4R immunoglobulin on TrpE-SalL4R fusion protein column (panel B). Binding of gold particles was not seen if the primary antibody was an irrelevant rabbit antisera (panel F). These data demonstrate that the SalL4R encoded proteins form part of the EEV envelope.

The significance of these findings are threefold. i) The virus is unable to spread efficiently in the absence of SalL4R gene expression. Thus a deletion mutant lacking SalL4R will be attenuated. ii) These observations identify the SalL4R gene as a site into which foreign DNA may be inserted into the virus genome. iii) It is known that the extracellular envelope proteins of the virus are the most important targets for neutralizing antibodies (Payne. L.G., J. Gen. Virol. 50, 89-100, 1980). Thus if an animal or human were vaccinated with a recombinant virus lacking the SalL4R gene, the immunity induced against vaccinia virus would be less potent than that induced by WT virus. Therefore upon re-vaccination the virus would replicate better and the immune response to foreign antigen(s) expressed by the virus used for revaccination would be stronger.

The applicants have provided herein sequence information for B15R, B18R and SalL4R, and identified the location of their nucleotide sequences in the viral

genome. Having done this, it is within the capability of one skilled in the art to either inactivate these sequences in or delete these sequences from the VV genome or change them to alter the function of the encoded protein product. All the necessary standard procedures are described in Molecular Cloning, eds. Sambrook, Fritsch and Maniatis, Cold Spring Harbour Laboratory Press 1989.

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Furthermore, it would be within the normal capability of one skilled in the art to develop useful pharmaceuticals and immunogens comprising the vaccinia virus vectors as herein provided; to use the immunogens to produce antibodies and the like; to use the antibodies in kits and pharmaceuticals; to isolate the identified sequences and use them in recombinant production methods.

Table 1

		B15R			B18R		
		1	2	3	1	2	3
B15R	1						
	2	6.71					
	3	1.63	2.44				
B18R	1	4.81	1.88	0.58			
	2	4.13	2.75	2.25	2.70		
	3	1.94	2.64	3.51	3.67	-0.21	
IL-1R Mouse	1	7.77	4.32	3.27	3.87	4.64	2.29
	2	4.70	7.62	2.20	0.39	6.42	3.11
	3	2.03	1.74	2.68	2.96	1.91	3.55
IL-1R Man	1	7.62	2.37	3.21	2.53	4.60	2.78
	2	4.07	8.29	1.27	0.31	5.88	3.80
	3	1.07	1.29	4.64	1.73	1.65	2.44
IL-6R Man		5.87	3.98	5.28	3.24	6.30	2.10
L1CAM	6	4.40	4.13	6.44	2.79	5.43	2.41
CHNCAM	1	4.12	4.01	4.07	3.19	5.54	3.28
MAG	3	4.04	4.67	6.17	1.22	3.10	3.96
PDGFR	3	2.84	2.81	5.14	3.88	4.86	3.24
TCRCD3		5.65	4.95	1.75	2.38	4.64	2.66
LAR	2	5.33	6.52	4.46	3.26	5.32	2.07
CEA	1	3.42	3.58	3.18	1.24	6.33	4.07
RPIgR	5	3.92	4.47	4.35	1.67	1.80	3.81

Table 1. Similarity scores for the Ig domains of vaccinia virus proteins B15R and B18R against selected domains from other Ig superfamily members computed using the ALIGN programme (Dayhoff, M.O., Barker, W.C. and Hunt, L.T. (1983). Meth. Enzymol. 91, 524-545). The programme compares to the best alignment score for two domains with the mean score of 100 alignments of the randomly scrambled sequences. The score for the best alignment of the real sequences is presented as the number of standard deviations from the mean score of the randomised sequences. Values of greater than 3.1 are significant (probability 10^{-3}), while values of 4.8, 6.0 and 7.9 indicate probabilities of 10^{-6}, 10^{-9} and 10^{-15}, respectively. The domains illustrated are from B15R amino acids 28-119 (1), 121-214 (2), 222-end (3): B18R 53-149 (1), 152-241 (2), 252-end (3); murine IL-1R precursor (Sims, J.E., March, C.J., Widmer, M.B., MacDonald, H.R., McMahan, D.J., Grubin, C.E., Wignall, J.M., Jackson, J.L., Call, S.M. Friend, D., Alpert, A.R. Gillis, S., Urdal, D.L. and Dower, S.K. (1988). Science 241, 585-589) 26-119 (1), 125-219 (2), 231-335 (3); human IL-1R (Sims, J.E., Acres, R.B., Grubin, C.E., McMahan, C.J., Wignall, J.M. (1989). Proc Natl. Acad. Sci USA 86, 8946-8950) 24-116 (1), 122-216 (2), 228-332 (3): human IL-6R (Yamasaki, K., Taga, T., Hirat, Y., Yawata, H., Kawanishi, Y., Seed, B., Taniguchi, T., Hirano, T and

Kishismoto, T. (1988). Proc. Jpn. Acad. 64, 209-211) 27-116; murine neural cell adhesion molecule L1 precursor (L1CAM) (Moos, M., Tacke, R., Scherer, H., Teplow, D., Freuth, K., Schachner, M. (1988). Nature 334, 701-703) 518-610 (6); chicken neural cell adhesion molecule (CHNCAM) (Hemperley, J.J., Murray, B.A., Edelman, G.M. and Cunningham, B.A. (1986). Proc. Natl. Acad. Sci. USA 83, 3037-3041) 2-97; myelin-associated glycoprotein (MAG) (Salzer, J.L., Holmes, W.P. and Colman, D.R. (1987). J. Cell Biol. 104, 957-965) 225-309; platelet derived growth factor receptor (PDGFR) (Yarden, Y., Escobedo, J.A., Kuang, W.J., Yang-Feng, T.L., Daniel, T.O., Tremble, P.M., Chen, E.Y., Ando, M.E., Harkins, R.N., Francke, U., Friend, V.A., Ullrich, A. and Williams L.T. (1986). Nature 323, 226-232) 183-279; T cell receptor CD3 epsilon chain (TCRCD3) (Clevers, H., Dunlap, S., Saito, H., Georgopoulos, K., Wileman, T. and Terhorst, C., (1988). Proc. Natl. Acad. Sci. USA 85, 8623-8627) 1-82; leukocyte antigen receptor protein (LAR) (Streuli, M., Krueger, N.X., Hall, L.R., Schlossman, S.F. and Saito, H. (1988). J. Exp. Med. 168, 1553-1562) 125-216; carcinoembryonic antigen precursor (CEA) (Oikawa, S., Nakazato, H. and Kosaki, G. (1987). Biochem. Biophys. Res. Commun. 142, 511-518) 113-201; rabbit poly-Ig receptor (RPIgR) (Mostov, K.E., Friedlander, M. and Blobel, G. (1984). Nature 308, 37-43) 458-558.

CLAIMS

1. A vaccinia virus vector wherein:

(a) part or all of one or more of the nucleotide sequences designated herein as (i) B15R (ii) B18R and (iii) SaIL4R is deleted from the viral genome; and/or

(b) one or more of said nucleotide sequences is inactivated to alter the function of a protein product encoded by said nucleotide sequence.

(c) one or more of said nucleotide sequences is changed to alter the function of a protein product encoded by said nucleotide sequence.

2. A recombinant virus vector which comprises a vaccinia virus vector according to claim 1 which comprises nucleotide sequences encoding one or more heterologous polypeptides.

3. A recombinant virus vector according to claim 2 wherein the heterologous polypeptide is an immunogen.

4. A recombinant virus vector according to claim 2 or claim 3 wherein the nucleotide sequences encoding one or more heterologous polypeptides is inserted into a ligation site created by the deletion of the nucleotide sequences B15R, B18R and SaIL4R.

5. A recombinant virus vector according to any one of claims 2 to 4 wherein the heterologous polypeptide is mammalian CD23.

6. A recombinant virus vector according to claim 5 wherein the heterologous polypeptide is human CD23.

7. A pharmaceutical which comprises a vaccinia virus vector according to claim 1, or a recombinant virus vector according to any one of claims 2 to 6.

8. A pharmaceutical according to claim 7 which is a vaccine.

9. A pharmaceutical according to claim 7 which is an immunotherapeutic.

10. A method which comprises the use of a vaccinia virus vector according to claim 1, or a recombinant virus vector according to any one of claims 2 to 6, in the preparation of a medicament for use as a vaccine or immunotherapeutic.

11. An immunogen which comprises a vaccinia virus vector according to claim 1, or a recombinant virus vector

according to any one of claims 2 to 6.

12. A method which comprises administering to a mammalian subject an immunogen according to claim 11 for the purpose of producing antisera, monoclonal and polyclonal antibodies or T. cells with specificity for polypeptides encoded by immunogen.

13. A binding substance comprising an antiserum, or monoclonal or polyclonal antibodies or T. cell obtainable by the method of claim 12 or derivatives and/or fragments of said antibodies with specificity for polypeptides encoded by the immunogen.

14. A diagnostic kit or device which comprises a binding substance according to claim 13.

15. A diagnostic method which comprises using a binding substance according to claim 13 or a diagnostic kit or device according to claim 14 to detect an analyte of interest in a clinical sample obtained from a mammalian subject.

16. A pharmaceutical which comprises a binding substance according to claim 13.

17. A polypeptide encoded by any of the nucleotide sequences designated herein as (i) B15R (ii) B18R and (iii) SaIL4R or a functionally equivalent fragment, allele, derivative or variant of said polypeptide.

18. A pharmaceutical which comprises a polypeptide according to claim 17 which is encoded by either of the nucleotide sequences designated herein as (i) B15R and (ii) B18R.

19. A pharmaceutical according to claim 18 which is an anti-inflammatory medicament.

20. A method which comprises the use of a polypeptide according to claim 17 which is encoded by either of the nucleotide sequences designated herein as (i) B15R and (ii) B18R, in the preparation of a medicament for treatment of inflammatory conditions.

21. A sub-genomic nucleotide sequence encoding a polypeptide according to claim 17.

22. A cloning vector or an expression vector comprising a sub-genomic nucleotide sequence according to claim 21.

23. A host cell containing a vector according to claim

22.

24. A cell culture comprising host cells according to claim 23 and capable of producing a polypeptide according to claim 16.

25. A method for attenuating a vaccinia virus vector which comprises:

(a) deleting part or all of one or more of the nucleotide sequences designated herein as (i) B15R; (ii) B18R and (iii) SaIL4R from the viral genome; and/or

(b) inactivating one or more of said nucleotide sequences by mutating the sequence or inserting foreign DNA; and/or

(c) changing one or more of said nucleotide sequences to alter the function of a protein product encoded by the nucleotide sequence.

Fig. 1A

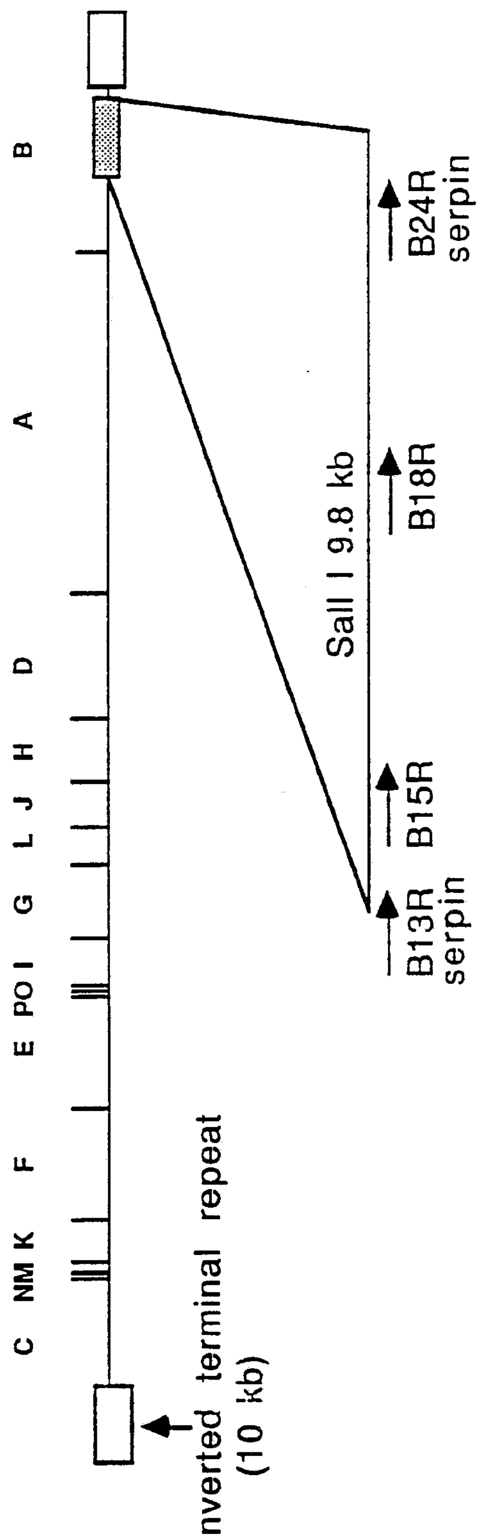
C
NM K
F
E
PO I
G
L
J
H
D
A
B
inverted terminal repeat
(10 kb)
SalI I 9.8 kb
B13R
serpin
B15R
B18R
B24R
serpin

Fig. 1B

```
CGTGGATTATAACATCATATTCCGGCGTATGAATTGATGAGTTGTACATCTTGACATTTTCTT

 M  S  I  L  P  V  I  F  L  S  I  F  F  Y  S  S  F  V  Q  T  F
ATGAGTATACTACCTGTTATATTTCTTTCTATATTTTTTTATTCTTCATTCGTTCAGACTTTT

 N  E  P  V  I  L  P  C  P  Q  I  N  T  L  S  S  G  Y  N  I  L
AACGAGCCAGTAATCTTACCATGTCCTCAAATAAATACGCTATCATCCGGATATAATATATTA

 G  S  N  M  L  I  L  N  P  T  Q  S  D  S  G  I  Y  I  C  I  T
GGTAGCAATATGCTAATTCTGAACCCGACACAATCAGACTCTGGTATTTATATATGCATTACC

 E  S  N  I  D  L  I  S  Y  P  Q  I  V  N  E  R  S  T  G  E  M
GAATCAAATATAGATCTTATCTCGTATCCACAAATAGTAAATGAGAGATCTACTGGCGAAATG

 S  G  H  R  R  L  R  N  K  R  L  K  Q  R  T  P  G  I  I  T  I
AGCGGGCATCGACGCCTTAGAAATAAGAGACTTAAACAACGGACACCTGGAATTATTACCATA

 G  G  K  T  Y  N  V  T  R  I  V  K  L  E  V  R  D  K  I  I  P
GGTGGCAAAACATATAACGTAACCAGAATTGTAAAATTAGAGGTACGGGATAAAATAATACCT

 A  C  R  V  S  L  R  P  P  T  T  D  A  D  V  F  W  I  S  N  G
GCATGCAGAGTATCGTTGAGACCTCCCACAACGGATGCAGACGTCTTTTGGATAAGTAATGGT

 I  Y  M  T  D  K  R  R  V  I  T  S  R  L  N  I  N  P  V  K  E
ATCTATATGACCGATAAGAGACGTGTTATTACATCCCGGTTAAACATTAATCCTGTCAAGGAA

 V  T  V  S  I  T
GTTACTGTTAGTATAACGTGAATGTATGTTGTTACATTTCCATGTCAATTGAGTTTATAAGAA
```

Fig. 1B cont.

```
CTTTCTTCTCTTCTCCCTTTCCCAGAAACAAACTTTTTTTACCCACTATAAAATAAA  120

 N  A  P  E  C  I  D  K  G  Q  Y  F  A  S  F  M  E  L  E    40
AACGCGCCTGAATGTATCGACAAAGGGCAATATTTTGCATCATTCATGGAGTTAGAA  240

 D  I  L  W  E  K  R  G  A  D  N  D  R  I  I  P  I  D  N    80
GATATTTTATGGGAAAAACGAGGAGCGGATAATGATAGAATTATACCGATAGATAAT  360

 T  N  E  T  Y  C  D  M  M  S  L  N  L  T  I  V  S  V  S   120
ACGAACGAAACCTACTGTGACATGATGTCGTTAAATTTGACAATCGTGTCTGTCTCA  480

 V  C  P  N  I  N  A  F  I  A  S  N  V  N  A  D  I  I  W   160
GTATGTCCCAATATTAATGCATTTATTGCTAGTAACGTAAACGCAGATATTATATGG  600

 E  D  V  R  K  N  D  A  G  Y  Y  T  C  V  L  E  Y  I  Y   200
GAAGATGTTAGAAAAAATGATGCTGGTTATTATACATGTGTTTTAGAATATATATAC  720

 S  T  M  Q  L  P  D  G  I  V  T  S  I  G  S  N  L  T  I   240
TCTACTATGCAATTACCAGATGGCATTGTAACTTCAATAGGTAGTAATTTGACTATT  840

 M  Y  Y  E  E  D  D  G  D  G  N  G  R  I  S  V  A  N  K   280
ATGTATTACGAAGAAGATGATGGGGACGGAAACGGTAGAATAAGTGTAGCAAATAAA  960

 E  D  A  T  T  F  T  C  M  A  F  T  I  P  S  I  S  K  T   320
GAAGATGCTACAACGTTTACGTGTATGGCGTTTACTATTCCTAGCATCAGTAAAACA 1080

                                                           326
TTTTTATACATTATCTTCCAACAAGCAATTGACGAACGTATTGCTATGATTAACTCC 1200
```

Fig.1C

```
GACAATTAACGATCTTTATAATATATCGTATCCACCTACCAAAGTATAGTTGTATTTTTCTCATGCGATGTGT

 M  T  M  K  M  M  V  H [I  Y  F  V  S  L  L  L  L  L  F] H  S  Y  A  I
ATGACGATGAAAATGATGGTACATATATATTTCGTATCATTATTGTTATTGCTATTCCACAGTTACGCCATAG

 L  P  A  K  D  S  K  W  L  N  P  A  C  M  F  G  G  T  M  N  D  I  A  A
CTACCAGCTAAAGACTCTAAATGGTTGAATCCAGCATGTATGTTCGGAGGCACAATGAATGATATAGCCGCTC

 L  S  H  R  Y  K  D  Y  V  V  K  W  E  R  L  E  K  N  R  R  R  Q  V  S
TTATCGCACAGATATAAAGACTATGTGGTTAAATGGGAAAGGCTAGAAAAAAATAGACGGCGACAGGTTTCTA

 K  F  S  N  R  R  Y  L  C  T  V  T  T  K  N  G  D  C  V  Q  G  I  V  R
AAATTCAGTAACCGTAGGTATTTGTGCACCGTAACTACAAAGAATGGTGACTGTGTTCAGGGTATAGTTAGAT

 G  T  H  D  K  Y  G  I  D  L  Y  C  G  I  L  Y  A  K  H  Y  N [N  I  T]
GGTACTCATGATAAGTATGGCATAGACTTATACTGTGGAATTCTTTACGCAAAACATTATAATAATATAACTT
             K                                               N
 Q  T  G  K  E  L  I  I  H  N  P  E  L  E  D  S  G  R  Y  D  C  Y  V  H
CAAACGGGAAAGGAATTAATTATTCATAATCCAGAGTTAGAAGATAGCGGAAGATACGACTGTTACGTTCATT

 I  L  T  V  I  P  S  Q  D  H  R  F  K  L  I  L  D  P  K  I [N  V  T] I
ATACTTACGGTTATACCGTCACAAGACCACAGGTTTAAACTAATACTAGATCCAAAAATCAACGTAACGATAG

 I  D  D  V  L  I  E  W  E  N  P  S  G  W  L  I  G  F  D  F  D  V  Y  S
ATTGACGATGTACTGATTGAATGGGAAAATCCATCCGGATGGCTTATAGGATTCGATTTTGATGTATACTCTG

[N  V  T] E  E  Y  I  G  N  T  Y  K  C  R  G  H  N  Y  Y  F  E  K  T  L
AATGTTACTGAAGAATATATAGGTAATACATATAAATGTCGTGGACACAACTATTATTTTGAAAAAACCCTTA

TTACTGAATAATTATTATTATTATTTATATCGTATTTGTGCTATAACGCGACTATCTAGGTATTTGTATCTCA
```

Fig.1C cont.

```
GTAAAAAAACTGATATTATATAAATATTTTAGTGCCGTATAATAAAG   120

D  I  E  N  E  I  T  E  F  F  N  K  M  R  D  T     40
ACATCGAAAATGAAATCACAGAATTCTTCAATAAAATGAGAGATACT   240

L  G  E  P  F  S  A  K  C  P  P  I  E  D  S  L     80
TAGGAGAGCCATTCAGCGCAAAGTGTCCTCCTATTGAAGACAGTCTT   360

N  K  R  V  K  H  G  D  L  W  I  A  N  Y  T  S    120
ATAAACGTGTTAAACATGGTGATTTATGGATAGCCAACTATACATCT   480
          K
S  H  I  R  K  P  P  S  C  I  P  K  T  Y  E  L    160
CTCATATTAGAAAACCTCCTTCATGCATTCCAAAAACATATGAACTA   600

W  Y  K  D  N  K  E  I  N  I  D  D  I  K  Y  S    200
GGTATAAAGATAATAAGGAAATTAATATCGACGACATTAAGTATTCA   720

Y  D  D  V  R  I  K  N  D  I  V  V  S  R  C  K    240
ACGACGACGTTAGAATCAAGAATGATATCGTAGTATCAAGATGTAAA   840

G  E  P  A  N  I  T  C  T  A  V  S  T  S  L  L    280
GAGAACCTGCCAATATAACATGCACTGCTGTGTCAACGTCATTATTG   960

V  L  T  S  R  G  G  I  T  E  A  T  L  Y  F  E    320
TTTTAACTAGTAGAGGCGGTATTACCGAGGCGACCTTGTACTTTGAA  1080

T  T  T  V  V  L  E                                351
CAACTACAGTAGTATTGGAGTAAATATACAATGCATTTTTATATACA  1200

CCGATAGAGAACATATAAAT                              1293
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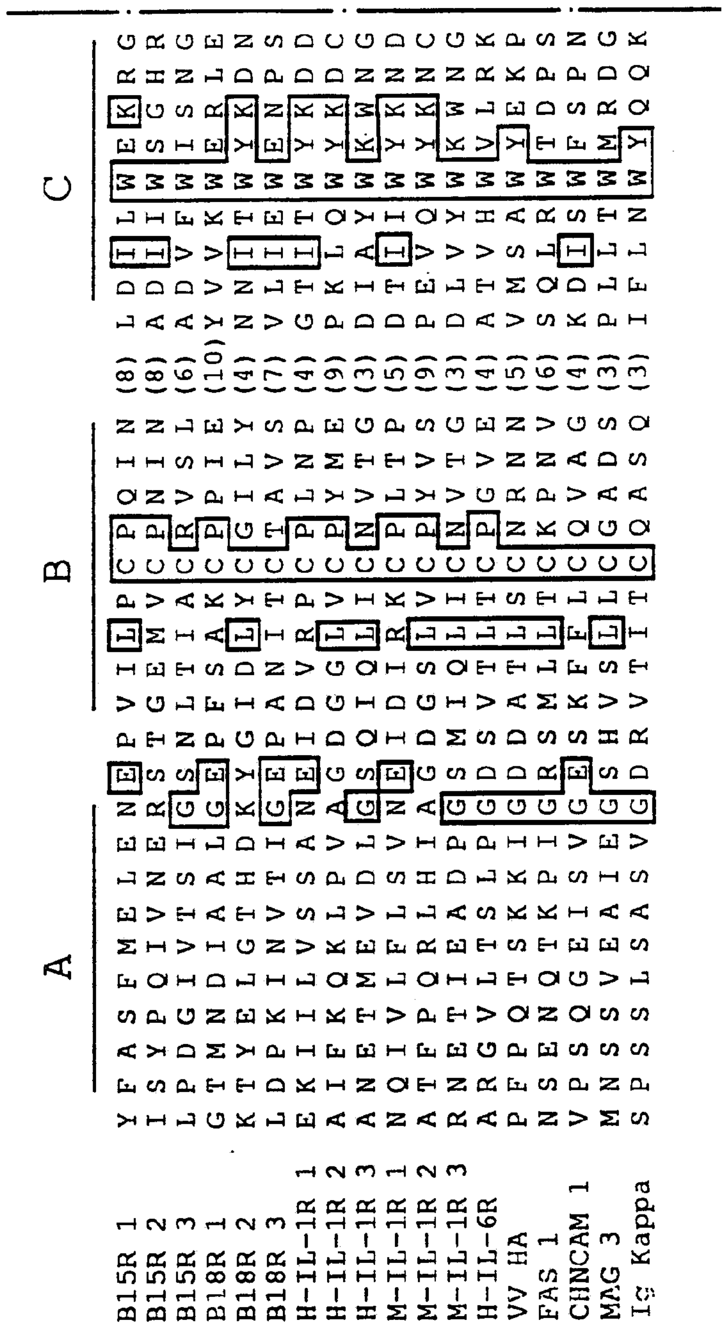
A
B
C
B15R 1
B15R 2
B15R 3
B18R 1
B18R 2
B18R 3
H-IL-1R 1
H-IL-1R 2
H-IL-1R 3
M-IL-1R 1
M-IL-1R 2
M-IL-1R 3
H-IL-6R
VV HA
FAS 1
CHNCAM 1
MAG 3
Ig Kappa

Fig.2.

Fig. 2 cont.

```
                  E                        F                      G
(12) S N M L I L N P T - - Q S D S G I Y I C I T T N E T Y C D M M S L N L T I
(10) T P G I I T I E D V R K N D A G Y Y T C V L E Y I Y G G K T Y N V T R I V
(29) T S R L N I N P V - K E E D A T T F T C M A F T I P S I S K T V T V S I T
(13) H G D L W I A N Y T S K F S N R R Y L C T V T T K N G D C V Q G I V R S H
(13) G K E L I I H N P - E L E D S G R Y D C Y V H Y D D V R I K N D I V V S R
(21) E A T L Y F E N V T E E Y I G N T Y K C R G H N Y Y F E K T L T T T V V L
(16) K E K L W F V P A - K V E D S G H Y Y C V V R N S S Y C L R I K I S A K F
(13) K D R L I V M N V - A E K H R G N Y T C H A S Y T Y L G K Q Y P I T R V I
(28) I T V L N I S E I E S R F Y K H P F T C F A K N T H G I D A A Y I Q L I Y
(16) N E H L W F V P A - K V E D S G Y Y Y C I V R N S T Y C L K T K V T V T V
(13) K D K L L V R N V - A E E H R G D Y I C R M S Y T F R G K Q Y P V T R V I
(28) I T T L N I S E V K S Q F Y R Y P F I C V V K N T N I F E S A H V Q L I Y
(13) G R R L L L R S V - Q L H D S G N Y S C Y R A G R P A G T V H L L V D V P
(32) V T T I T I K S L - T A R D A G T Y V C A F F M T S T T N D T D K V D Y E
(27) V L T L M I P E L - R E A D T G T Y T C S A L Y S N T K Q L S K S V H V R
(19) S S T L T I Y N A - N I D D A G I Y K C V V S S V E E G D S E A T V N V K
(9)  S L Y L D L E E V - T P A E D G I Y A C L A E N A Y G Q D N R T V E L S V
(30) D F T F T I S S L - Q P E D I A T Y Y C Q Q F D N L P L T F G G G T K V D
```

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Fig.3.

```
IL1R-H     MKVLLRLICFIALLISSLEADKCKEREEKIILVSS-ANEIDVRPCPLNPNE-----HKGTITWYKDDSKTPVSTEQASRIHQHKE
IL1R-M   MENMKVLLGLICLMVPLLS-LEIDVCTEYPNQIVLFLS-VNEIDIRKCPLTPNKM----HGDTIIWYKNDSKTPISADRDSRIHQQNE
B15R     MSILPVIFLSIFFYSSFVQTFNAPECIDKGQYFASFMELENEPVILPCP-QINTLSSGYNILDILWEK----RGADNDRIIPIDNGSN
IL6R-H   MLAVGCAI-IAALLAAPGAALAPRRCPAQEVARGVLTSLPGDSVTLTCP-GVEPE----DNATVHWVL---RKPAAGSHPSRWAGMGR

IL1R-H   KIWFVPAKVEDSGHYYCVVRNSSYCLRIKISAKFVENEPNLCYNAQAIFKQKLPVAGDGGLVCPYMEFFKNENNELPKLQWYKDCKPL
IL1R-M   HLWFVPAKVEDSGYYYCIVRNSTYCLKTKVTVTVLENDPGLCYSTQATFPQRLHIAGDGSLVCPYVSYFKDENNELPEVQWYKNCKPL
B15R     MIILNPTQ-SDSGIYICITTNETYCDMMSLNLTIVSVSES--NIDLISYPQIVNERSTGEMVCPNINAFI-ASNVNADIIWSGHRR--
IL6R-H   RLLLRSVQLHDSGNYSCYRAGRPAGTVHLLVDVPPE-EPQL

IL1R-H   LIDNTHFSGVKDRLI-VMNVAEKHRGNYTCHASYTYLGKQYPITRVIEFITLEENKPTRPVIVSPANETMEVDLGSQIQLICNVTGQL
IL1R-M   LLDNVSFFGVKDKLL-VRNVAEEHRGDYICRMSYTFRGKQYPVTRVIQFITIDENKRDRPVILSPRNETIEADPGSMIQLICNVTGQF
B15R     -LRNKRLKQRTPGIITIEDVRKNDAGYYTCVLEYIYGGKTYNVTR-IVKLEV-RDKIIPSTMQLPDG--IVTSIGSNLTIACRVSLRP

IL1R-H   ----SDIAYWKWNGSVIDEDDPVLGEDYYSVENP--ANKRRSTLIT-VLNISEIESRFYKHPFTCFAKNTHGIDAAYIQLIYPVTN
IL1R-M   ----SDLVYWKWNGSEIEWNDPFLAEDYQFVEHP--STKRKYTLIT-TLNISEVKSQFYRYPFICVVKNTNIFESAHVQLIYPVPD
B15R     PTTDAD-VFWISNGMYYEEDDGDGNGRISVANKIYMTDKRR--VIISRLNINPVKEE-DATTEICMAFTIPSI-SKTVTVSIT
```

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Fig.4.

TAGGGATAGGAGTAGGAATGCCACAAATGAAAAAAATACTTAAAATGTAATCTTAATCGAGTACACCACACGACA

M N K H K T D Y A G Y A C C V I C G L I V G I I F
ATGAACAAACATAAGACAGATTATGCTGGTTATGCTTGCTGCGTAATATGCGGTCTAATTGTTGGAATTATTTTT

T A T L L K V V E R K L V H T P S I D K T I K D A
ACAGCGACACTATTAAAAGTTGTAGAACGTAAATTAGTTCATACACCATCAATAGATAAAACGATAAAAGATGCA

Y I R E D C P T D W I S Y N N K C I H L S T D R K
TATATTAGAGAAGATTGTCCTACTGACTGGATAAGCTATAATAATAAATGTATCCATTTATCTACTGATCGAAAA

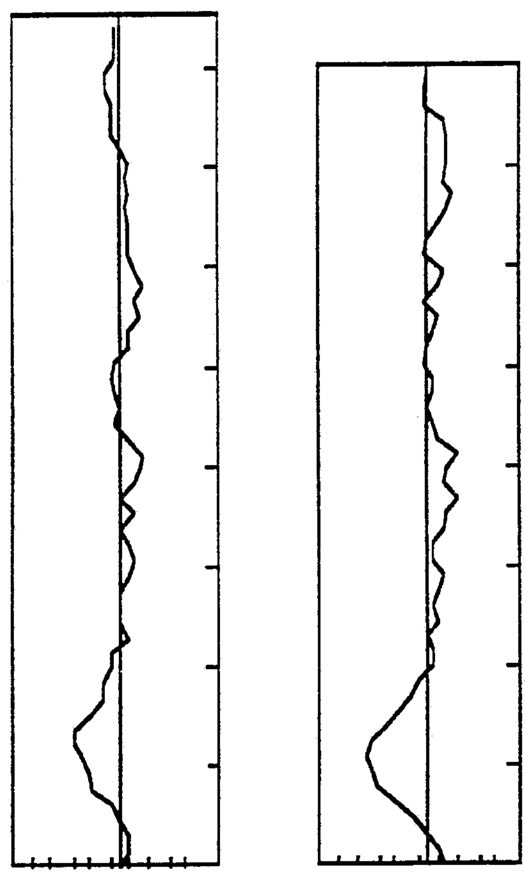
T W E E G R N A C K A L N P N S D L I K I E T P N
ACCTGGGAGGAAGGACGTAATGCATGCAAAGCTCTAAATCCAAATTCGGATCTAATTAAGATAGAGACTCCAAAC

E L S F L R S I R R G Y W V G E S E I L N Q T T P
GAGTTAAGTTTTTAAGAAGCATTAGACGCGGATATTGGGTAGGAGAATCCGAAATATTAAACCAGACAACCCCA

Y N F I A K N A T K N G T K K R K Y I C S T T N T
TATAATTTTATAGCTAAGAATGCCACGAAGAATGGAACTAAAAAACGGAAATATATTTGTAGCACAACGAATACT

P K L H S C Y T I
CCCAAACTGCATTCGTGTTACACTATATAACAATTACACTACATTTTTATCATACCACTACT

Fig. 5.



SALL4R

SALF3R

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Fig. 6.

```
ABLec  101    PGNLD
HFcR   101 RLKSQDLELSWNLNGLQADLSSFKSQELNERNEASDLLERLREE
FPV 8  101 PPVPPDIKILY
AFP    101 ATEAGPVSQRAPPN
FPV 2  101 VVVTCKWYYAFP
VV F3R 101 PSIDKTIKDAY
FPV 11 101 RYLVR
VV L4R 101 TFLHYKEELM

ABLec 201 ATIQSQLENAFISETVSNNRLWIGLNDIDLEGHYVWSNGEATDFT
HFcR   201 VSIHSPEEQDFLTKHASHTGSWIGLRNLDLKGEFIWVDGSHVDY
FPV 8  201 TSISSKEEFKFILRYKGPGNHWIGIEKVDFNGT
AFP    201 ASIHSQEEHSFI QTLNAGVVWIGGSACLQAGAWTWSDGTPMNF
FPV 2  201 IRFDNIETLNFVSRY GKGSYWIDINQNRKIPGINFSLYYEQGV
VV F3R 201 IKIETPNELSELRSI   RRGYWVGESEILNQTT      PYNF
FPV 11 201 NNININTNSGLLNT    SKDYWIKIVDELDCTN   INMCNFLY
VV L4R 201 PRPDTRHLRVLFSIF   YKDYWVSLKKTNDKWLDI NNDKDIDI
```

Fig. 6 cont.

```
                  WQEYDGHCYWASTYQVRWNDAQLACQTVHPGAYL
VTKLRMELQVSSGFVCNTCPEKWINFQRKCYYFGKGTKQWVHARYACDDMEGQ  L
                  CKEGWVGYNKNCYFFSEEKNNKSLAVERCKDMDGH  L
                  CPAGWQPLGDRCIYYETTAMTWALAETNCMKLGGH  L
             YFSKVCPDEWIGYNSKCYYFTINETNWNDSKKLCDVMDSS  L
              IREDCPTDWISYNNKCIHLSTDRKTWEEGRNACKALNPNSDL
                  CGKDWLEFDNICYFISENKLSWDDSMMVCDNLGGG
              PSACANGWIQYDKHCYLDTNIKMSTDNAVYQCRKLRAR  L

YWSSNNP NNW ENQDCGVVNYDTVTGQWDDDDCNKN KNFLCKMPIIGCPPCGI
SNWAPGEP TSRSQGEDCVMMR   GSGRWNDAFCDRKLGAWVCDRLATCTPPASE

RSWCSTKP DD  VLAACCMQMTAAADQCWDDLPCPASHKS VCAMTF
NDICLLFD TSNIIEMSCIFHE   RTICVKEDRYTHWYTEYMR
IAKNATKN GTKKRKYICSTTNTPKLHSCYTI
SNIVGCDI CTIEKFYICIKPINKINLFSYFVEYTK
SKLTNFKQLNSTTDAEACYIYKSGKLVKTVCKSTQSVLCVKKFYK
```

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Fig. 7.

```
TGATGGTAATCCAATTACAAAAACTACATCCGATTATCAAGATTCTGATGTATCACAAGAAGTTAG

 M  K  S  L  N  R  Q  T  V  S  R  F  K  K  L  S  V  P  A  A  I  M
ATGAAATCGCTTAATAGACAAACTGTAAGTAGGTTTAAGAAGTTGTCGGTGCCGGCCGCTATAATG
***
 E  L  M  P  S  A  C  A  N  G  W  I  Q  Y  D  K  H  C  Y  L  D  T
GAACTGATGCCTAGTGCTTGCGCCAATGGATGGATACAATACGATAAACATTGTTATTTAGATACT

 R  L  P  R  P  D  T  R  H  L  R  V  L  F  S  I  F  Y  K  D  Y  W
AGATTGCCTAGACCGGATACTAGACATCTGAGAGTATTGTTTAGTATTTTTTATAAAGATTATTGG

 I  D  I  S  K  L  T  N  F  K  Q  L  N  S  T  T  D  A  E  A  C  Y
ATAGATATTAGTAAATTAACAAATTTTAAACAACTAAACAGTACGACGGATGCTGAAGCGTGTTAT

 L  C  V  K  K  F  Y  K
CTATGTGTTAAAAAATTCTACAAGTGACAACAAAAAATGAATTAATAATAAGTCGTTAACGTACGC

TACATTGTATGATGATCTCGATAT
```

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Fig. 7cont.

```
AAAGTATTTTTGTGTTAAAACAATGAACTAATATTTATTTTTGTACATTAATAA
                                                     *
 M  I  L  S  T  I  I  S  G  I  G  T  F  L  H  Y  K  E
ATGATACTCTCAACCATTATTAGTGGCATAGGAACATTTCTGCATTACAAAGAA

 N  I  K  M  S  T  D  N  A  V  Y  Q  C  R  K  L  R  A
AACATTAAAATGTCTACAGATAATGCGGTTTATCAGTGTCGTAAATTACGAGCC

 V  S  L  K  K  T  N  D  K  W  L  D  I  N  N  D  K  D
GTAAGTTTAAAAAAGACCAATGATAAATGGTTAGATATTAATAATGATAAAGAT

 I  Y  K  S  G  K  L  V  K  T  V  C  K  S  T  Q  S  V
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CGCCATGGACGCCGCGTTTGTTATTACTCCAATGGGTGTGTTGACTATAACAGA
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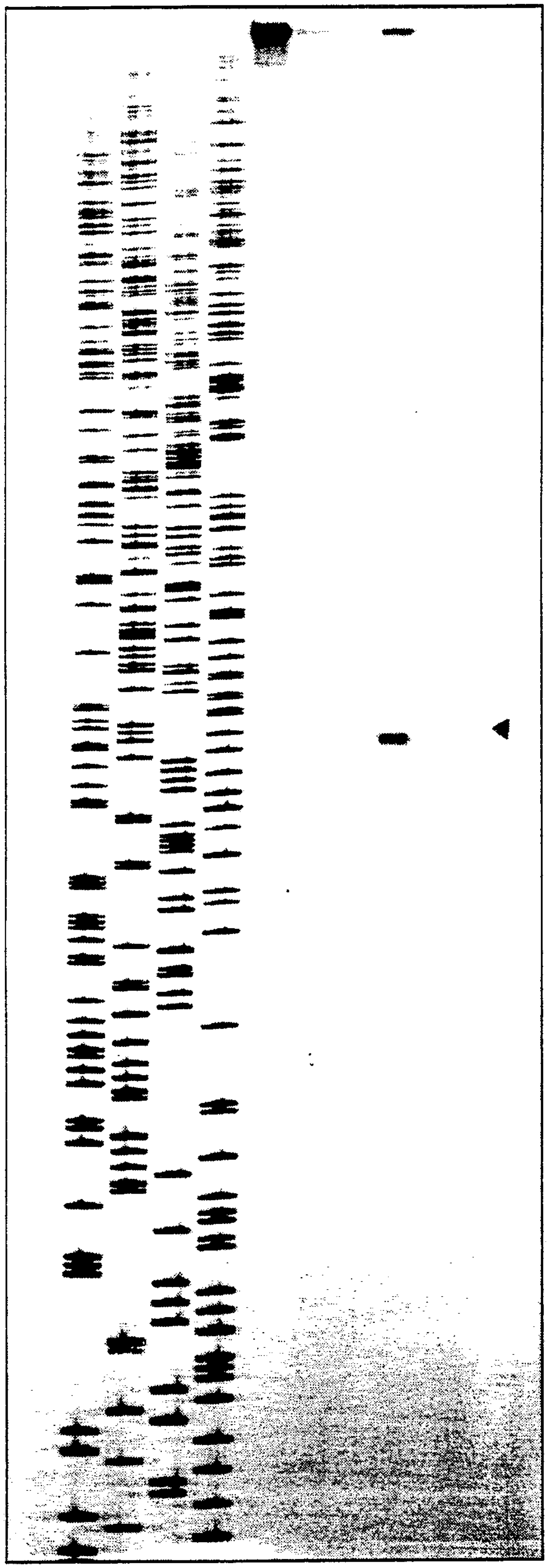


Fig. 8A.

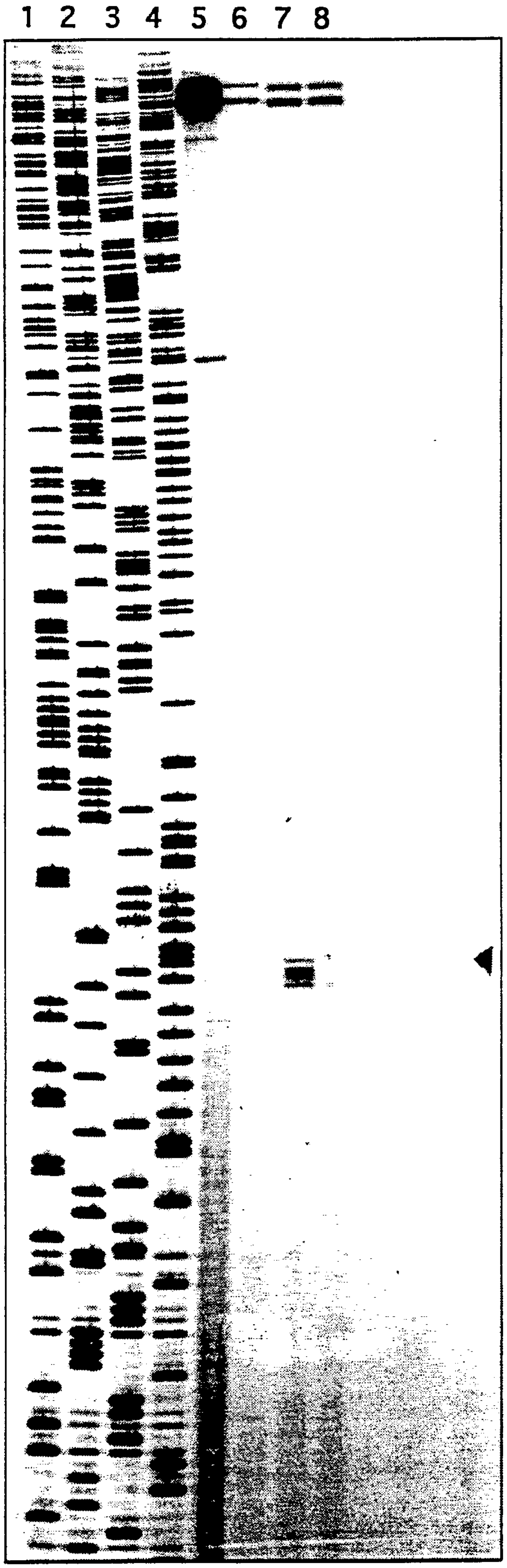
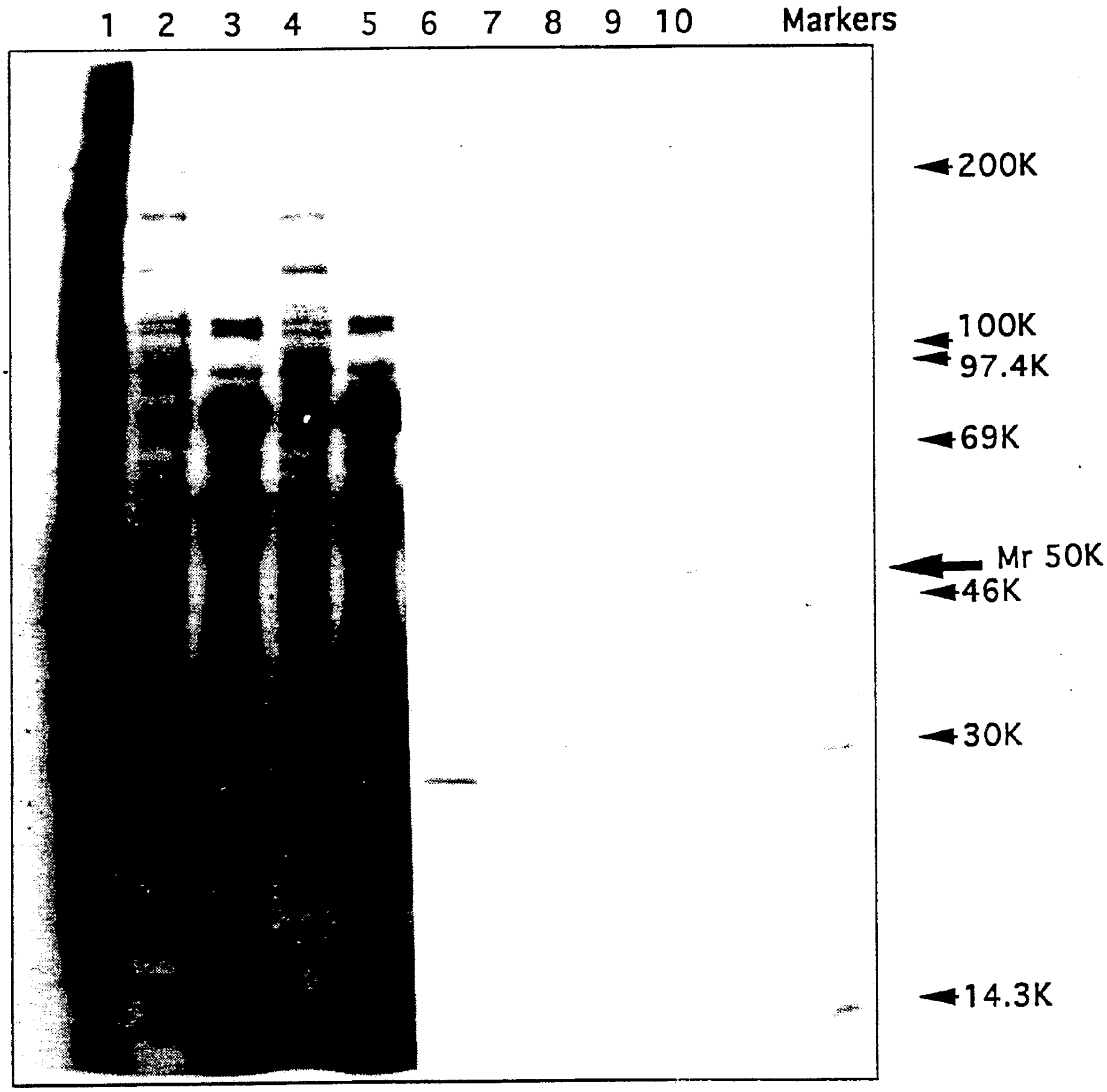


Fig. 8B.

Fig. 9.



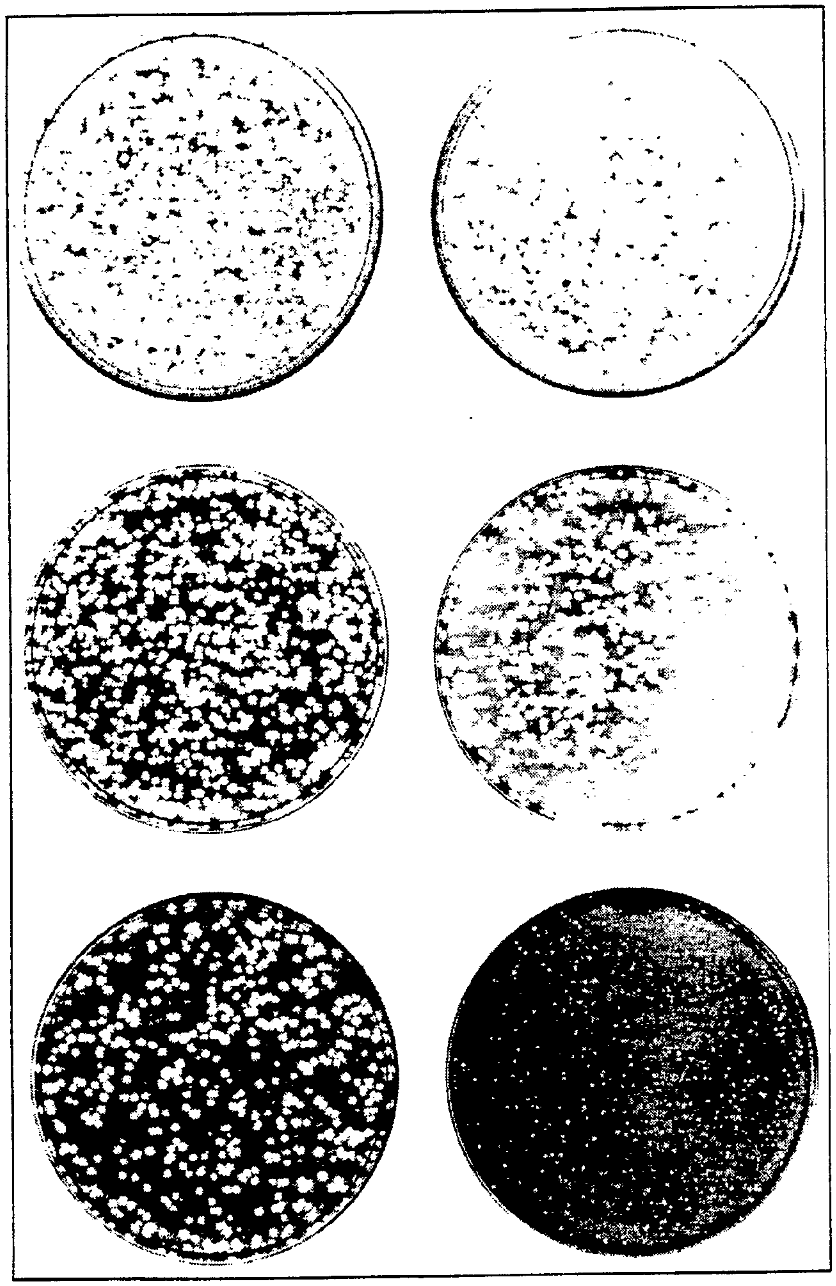

Inducible SalL4R
Endogenous SalL4R
+ IPTG
-IPTG
vSAD9
vSAD7
WR
+ + -
- + +

Fig. 10

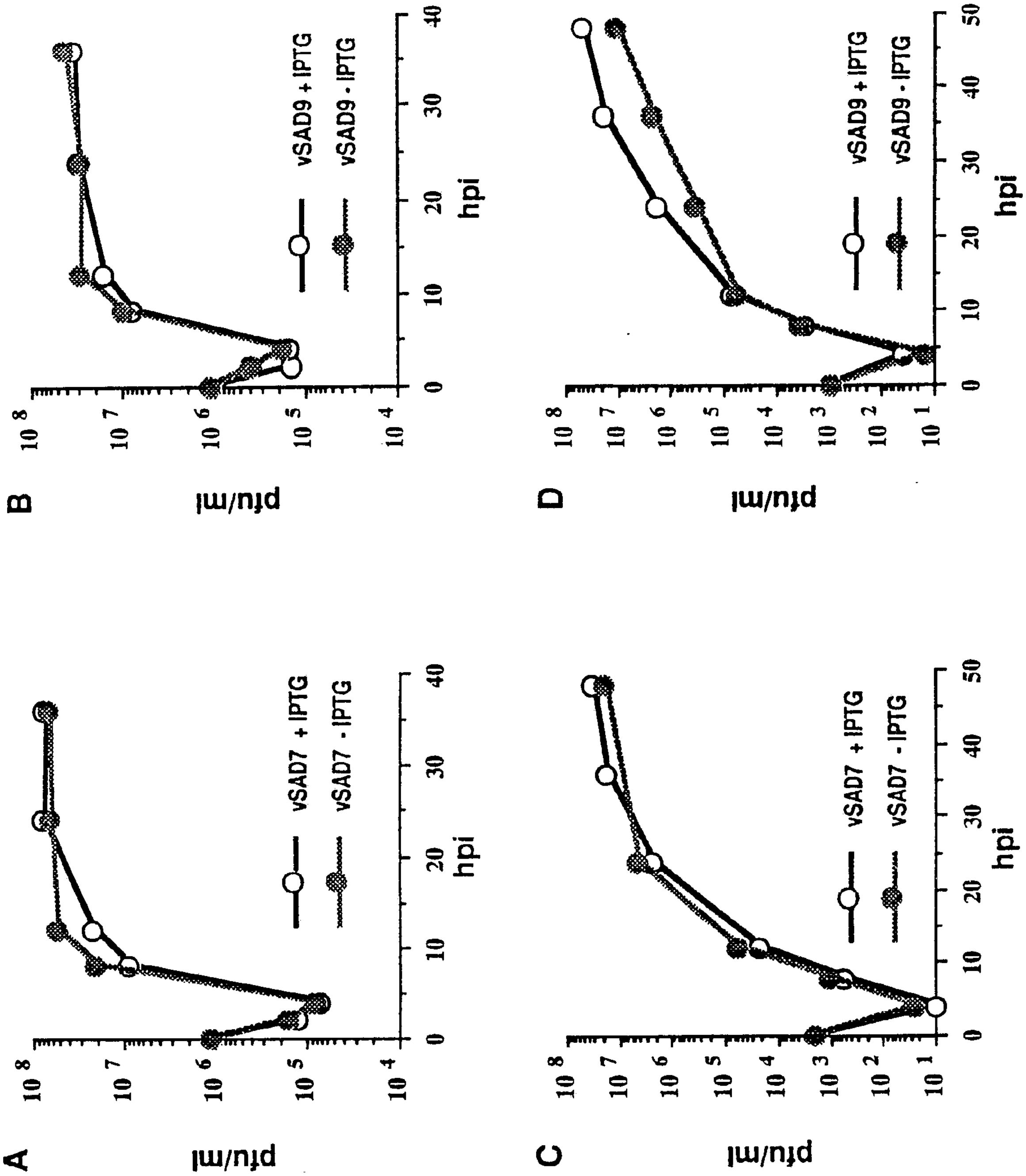


Fig.11.

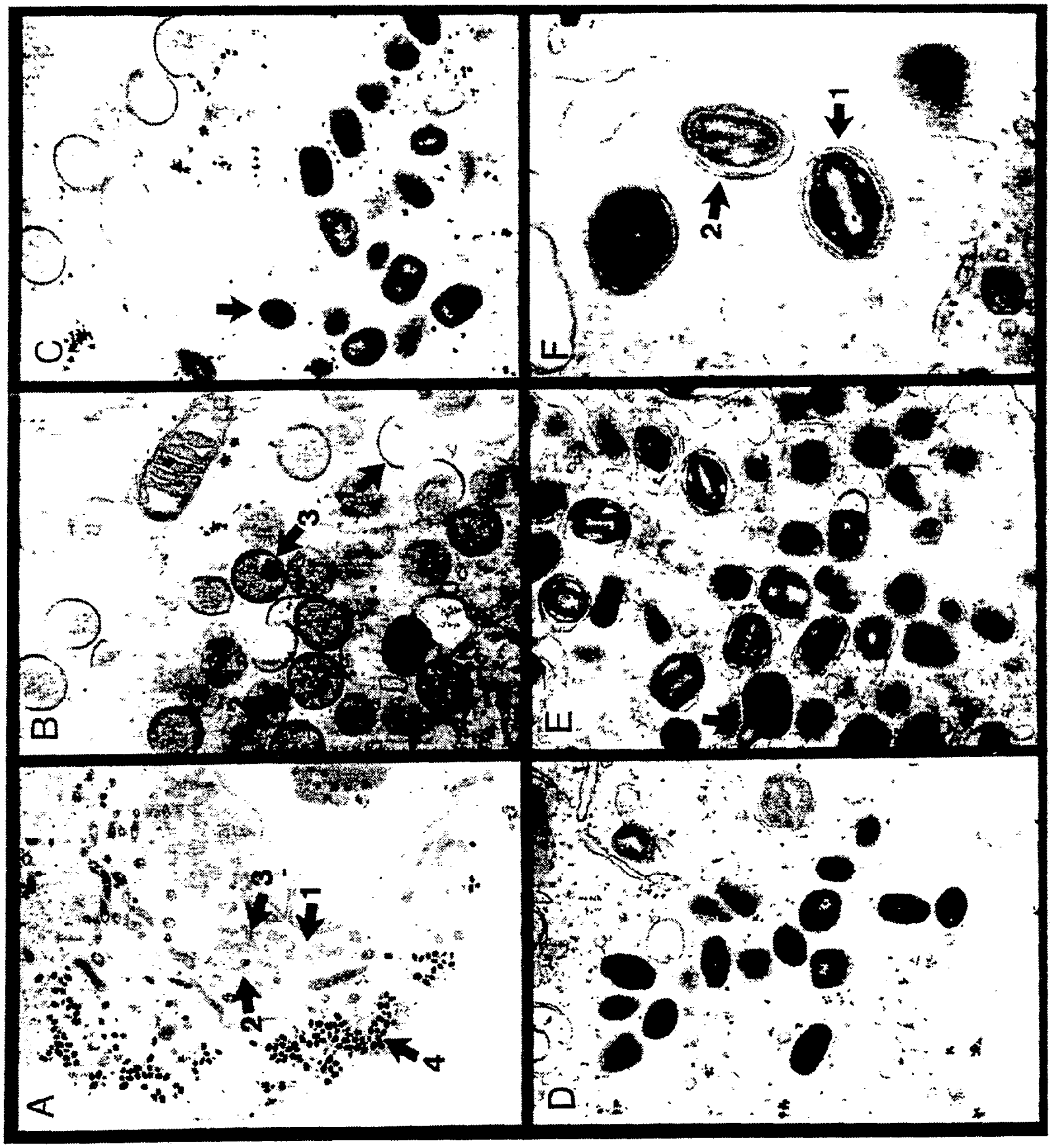


Fig. 12

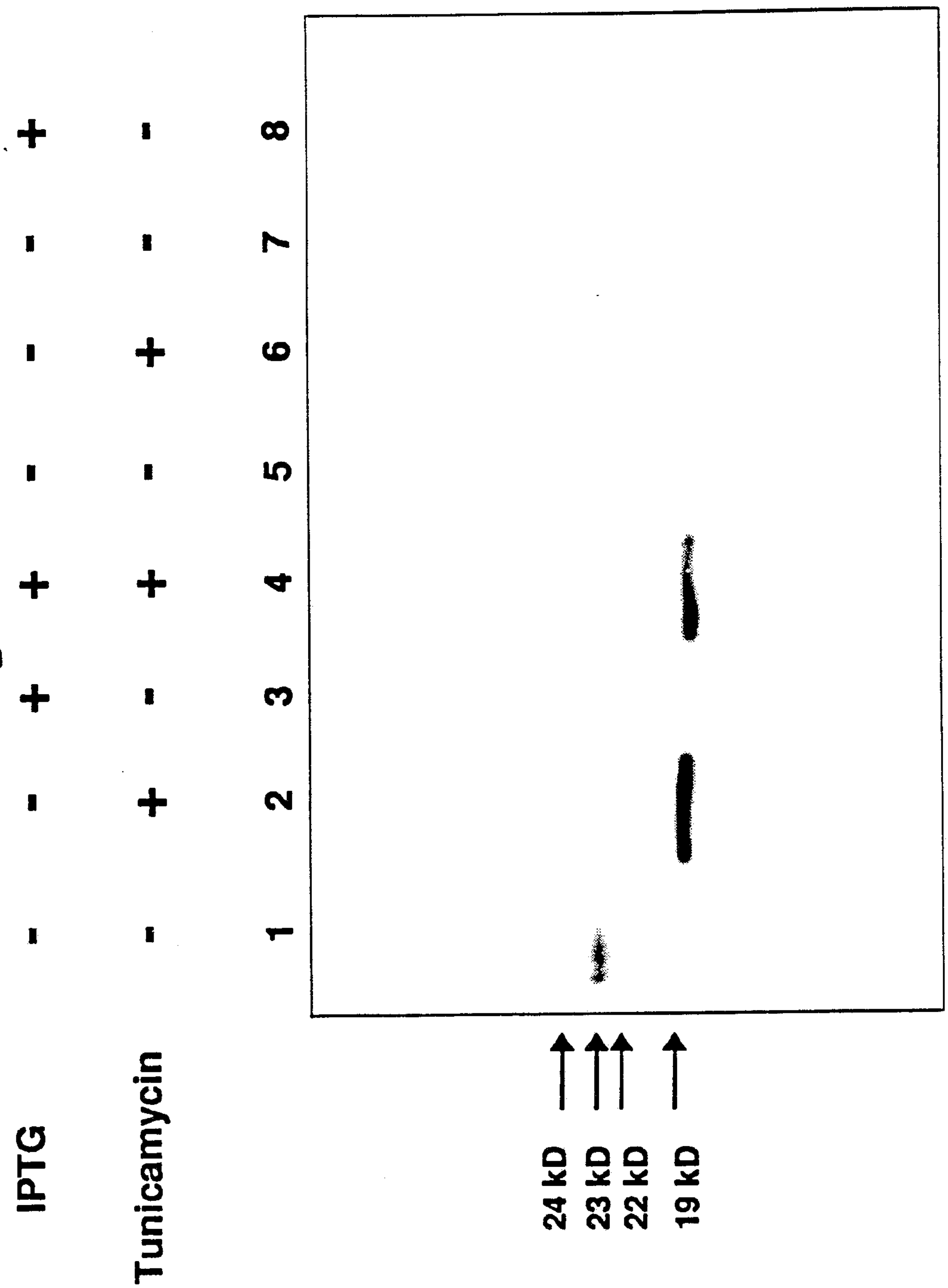


Fig. 13.

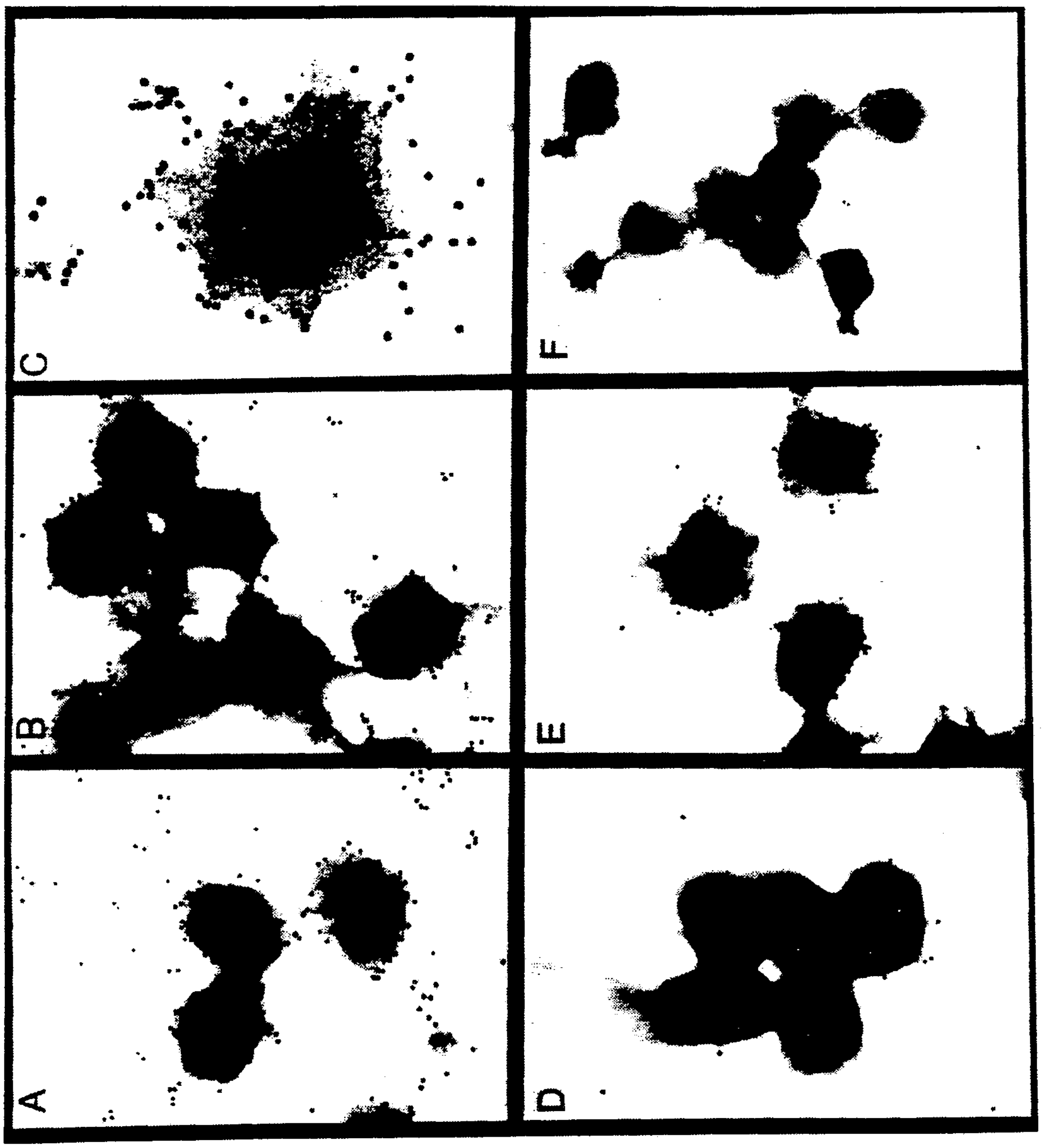


Fig. 14

INTERNATIONAL SEARCH REPORT

International Application No PCT/GB 91/01882

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl. 5 C12N15/86; A61K39/285; C07K13/00; C12N15/39
C12N5/10; C12N7/04

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl. 5	C12N ; C07K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category°	Citation of Document,[11] with indication, where appropriate, of the relevant passages[12]	Relevant to Claim No.[13]
P,X	JOURNAL OF GENERAL VIROLOGY vol. 72, no. 3, March 1991, READING, UK pages 511 - 518; SMITH, G.L. & CHAN, Y.S.: 'Two vaccinia virus proteins structurally related to the interleukin-1 receptor and the immunoglobulin superfamily' see the whole document ---	17,21, 22,23
P,X	VIROLOGY vol. 179, no. 1, November 1990, pages 247 - 266; GOEBEL, S.J. ET AL.: 'The complete DNA sequence of Vaccinia virus' see page 259; table 1 ---	17,21

-/--

° Special categories of cited documents :[10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
10 FEBRUARY 1992	13. 02. 92
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	CHAMBONNET F.J.

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		
Category °	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
X	VIROLOGY vol. 177, no. 1, September 1990, pages 588 - 594; UEDA, Y. ET AL.: 'Identification and nucleotide sequence of the gene encoding a surface antigen induced by vaccinia virus' cited in the application see the whole document ---	17,21
C,X	JOURNAL OF VIROLOGY vol. 28, no. 3, December 1978, USA pages 828 - 834; MCCRAE, M.A. & PENNINGTON, T.H.: 'Specific secretion of polypeptides from cells infected with vaccinia virus' see page 833 ---	17
P,X	VIROLOGY vol. 180, no. 1, January 1990, pages 406 - 410; PERKUS, M.E. ET AL.: 'Deletion of %% open reading frames from the termini of vaccinia virus' see the whole document ---	1,25